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Policies and Guidelines for Alaska Fish and Shellfish Health and Disease Control

Third Edition

by

Ted Meyers

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Alaska Department of Fish and Game **Division of Commercial Fisheries**

Symbols and Abbreviations

The following symbols and abbreviations, and others approved for the Système International d'Unités (SI), are used without definition in the following reports by the Divisions of Sport Fish and of Commercial Fisheries: Fishery Manuscripts, Fishery Data Series Reports, Fishery Management Reports, Special Publications and the Division of Commercial Fisheries Regional Reports. All others, including deviations from definitions listed below, are noted in the text at first mention, as well as in the titles or footnotes of tables, and in figure or figure captions.

Weights and measures (metric)

centimeter	cm
deciliter	dL
gram	g
hectare	ha
kilogram	kg
kilometer	km
liter	L
meter	m
milliliter	mL
millimeter	mm

Weights and measures (English)

cubic feet per second	ft^3/s
foot	ft
gallon	gal
inch	in
mile	mi
nautical mile	nmi
ounce	oz
pound	lb
quart	qt
yard	yd

Time and temperature

day	d
degrees Celsius	°C
degrees Fahrenheit	°F
degrees kelvin	K
hour	h
minute	min
second	s

Physics and chemistry

all atomic symbols	
alternating current	AC
ampere	A
calorie	cal
direct current	DC
hertz	Hz
horsepower	hp
hydrogen ion activity (negative log of)	pH
parts per million	ppm
parts per thousand	ppt, ‰
volts	V
watts	W

General

Alaska Administrative Code	AAC
all commonly accepted abbreviations	e.g., Mr., Mrs., AM, PM, etc.
all commonly accepted professional titles	e.g., Dr., Ph.D., R.N., etc.
at	@
compass directions:	
east	E
north	N
south	S
west	W
copyright	©
corporate suffixes:	
Company	Co.
Corporation	Corp.
Incorporated	Inc.
Limited	Ltd.
District of Columbia	D.C.
et alii (and others)	et al.
et cetera (and so forth)	etc.
exempli gratia (for example)	e.g.
Federal Information Code	FIC
id est (that is)	i.e.
latitude or longitude	lat. or long.
monetary symbols (U.S.)	$, ¢
months (tables and figures): first three letters	Jan,...,Dec
registered trademark	®
trademark	™
United States (adjective)	U.S.
United States of America (noun)	USA
U.S.C.	United States Code
U.S. state	use two-letter abbreviations (e.g., AK, WA)

Measures (fisheries)

fork length	FL
mideye-to-fork	MEF
mideye-to-tail-fork	METF
standard length	SL
total length	TL

Mathematics, statistics

all standard mathematical signs, symbols and abbreviations	
alternate hypothesis	H_A
base of natural logarithm	*e*
catch per unit effort	CPUE
coefficient of variation	CV
common test statistics	(F, t, χ^2, etc.)
confidence interval	CI
correlation coefficient (multiple)	R
correlation coefficient (simple)	r
covariance	cov
degree (angular)	°
degrees of freedom	df
expected value	*E*
greater than	>
greater than or equal to	≥
harvest per unit effort	HPUE
less than	<
less than or equal to	≤
logarithm (natural)	ln
logarithm (base 10)	log
logarithm (specify base)	$\log_2$, etc.
minute (angular)	'
not significant	NS
null hypothesis	H_O
percent	%
probability	P
probability of a type I error (rejection of the null hypothesis when true)	α
probability of a type II error (acceptance of the null hypothesis when false)	β
second (angular)	"
standard deviation	SD
standard error	SE
variance	
population	Var
sample	var

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POLICIES AND GUIDELINES FOR ALASKA FISH AND SHELLFISH HEALTH AND DISEASE CONTROL

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ABSTRACT

The 2003, 2010 and now 2014 revisions made in this document have not altered the intent, principals or rationale on which the fish disease policy was originally formulated by the State Pathology Review Committee in 1987 (Meyers et al. 1988). The changes made include (1) omission of the suggested regulation changes which were adopted by the Commissioner of the Department of Fish and Game and codified into state regulations in 2011, (2) editorial improvements with additional clarifications where necessary, (3) omission of the Sockeye Culture Policy and Diagnostic Procedures sections that were published as separate documents, (4) updating of the Shellfish Culture section, (5) updating of the current drug usage in aquaculture section, (6) addition of new publications in the Appendix. Minor revision was also made in the sections added in 2010 describing the responsibilities of the Alaska Department of Fish and Game's Fish Pathology Section, good fish culture practices to reduce disease, recognition of disease at the hatchery, the partial lists of finfish and shellfish pathogens in Alaska, and the investigation of fish kills. This revised document better reflects the current fish health program in Alaska.

Key words: fish, finfish, shellfish, regulation, policies, guidelines, health, disease, pathogen, culture, drug, aquaculture, hatchery

INTRODUCTION

This document includes the working statewide policies and recommendations used by recognized authorities and user groups for maintaining adequate finfish and shellfish health within the State of Alaska. These criteria include evaluation protocols for regulating and permitting fish transports, prophylactic measures and therapeutic treatments for infectious diseases of salmonid fishes and shellfish species. The criteria are established for the purpose of regulating interstate and intrastate transports of live finfish and shellfish or their gametes for transplanting into state waters, research and education purposes, and other interests not defined herein. The objective of this document is to prevent dissemination or amplification of infectious finfish and shellfish diseases within or outside the borders of Alaska without introducing impractical constraints for aquaculture and necessary stock renewal programs while maintaining other established state criteria regarding genetic and aquaculture policies. Additional fish culture and fish/shellfish health information is included in this document to provide further perspective on the current fish health program in Alaska.

CHANGES IN EXISTING REGULATIONS

The original recommendations for changes to existing regulations made by this committee, last published in the 2010 revision, have since been adopted by the commissioner of the Department of Fish and Game under a delegation of authority issued by the Board of Fisheries and codified into state regulations in Title 5 of the Alaska Administrative Code in February 2011.

The regulations may be accessed on the internet at the following link for Title 5 in the Fish and Game code, Part 1, Commercial and Subsistence Fishing and Private Nonprofit Salmon Hatcheries, Chapter 41: Transportation, Possession and Release of Live Fish; Aquatic Farming.

http://www.legis.state.ak.us/basis/folioproxy.asp?url=http://wwwjnu01.legis.state.ak.us/cgi-bin/folioisa.dll/aac/query=[JUMP:'Title5Chap41!2C+a!2E+3']/doc/{@1}?firsthit

TRANSPORT APPLICATIONS FOR FINFISH

The State of Alaska has large areas of separated watersheds supporting wild fish stocks that have never been examined for disease agents. Consequently, there is a risk of unknowingly transporting presently undiscovered (in Alaska) finfish disease agents from one major geographic area to another that may not be detected at the 5% level in 60 adult fish examined prior to transport. This risk will be minimized by the department's policy to discourage the transport of wild finfish stocks between the major geographic zones designated as Southeast, Kodiak Island, Prince William

Sound, Cook Inlet, Bristol Bay, Arctic-Yukon-Kuskokwim, and Interior. To maintain consistency with the Alaska Department of Fish and Game (ADF&G) Genetics Policy, and because wild fish stocks are in several hatchery water supplies, this disease policy will also include hatchery stocks of fish, with exceptions considered only on a case-by-case basis. Proposals to do so must be for gametes only and accompanied by adequate justification for using a nonlocal stock. There also must be a hatchery disease history for cultured fish that demonstrates no detectable disease agents of transport significance for the last two consecutive years of screening a minimum of 150 adult broodfish and no detection of such agents in progeny fish (Tables 1–4).

Table 1. –Wild fish transplants.

A. Wild fish transplants	Disease considerations
1. Between watersheds within a designated geographic area	
a. Transplant of adult fish to a watershed barren of salmonids	• Prior year sampling recommended to define year-to-year variability of disease prevalence. • Sampling required in same year but prior to transplant of the adult fish stock. Class II disease criteria:[a] 1. Bacterial Kidney Disease (BKD): Agent *Renibacterium salmoninarum* (Rs) cannot exceed levels in Schedule I (See Appendix A). 2. Furunculosis: Carrier state cannot exceed levels in Schedule I. 3. Infectious Hematopoietic Necrosis Virus (IHNV): No samples required unless proposed transplants are IHNV-susceptible salmonids from a sockeye or kokanee watershed since IHNV has not been prevalent in salmonid species other than anadromous sockeye salmon. All sockeye salmon and most kokanee stocks are presumed carriers. Detection of IHNV in any salmonid other than sockeye/kokanee precludes use for transplant. 4. *Ichthyophthirius* (ICH): Not applicable unless present as a clinical disease, in which case consideration would be on a case-by-case basis. 5. Enteric Redmouth (ERM): An infrequent disease in Alaska caused by *Yersinia ruckeri* (Types 1 & 2). Its dissemination is a significant concern when detected. If diagnosed, transplant of those fish would be decided on a case-by-case basis.
b. Transplant of juvenile fish to a watershed barren of salmonids.	Class II disease criteria: 1. BKD: No significant (0.5% per day) mortality and immediate disease history of hatchery performance cannot exceed levels in Schedule I. 2. Furunculosis: Indicated by fluorescent antibody test (FAT) with confirmation by isolation. If the disease state exists, fish must be treated for release when mortality is insignificant and prevalence does not exceed Schedule I. If prevalence of infection exceeds Schedule I, fish cannot be released. Withdrawal period after drug therapy may be required. 3. IHNV (sockeye, kokanee): Release if no disease. Clinical signs of IHN or detection of virus will require destruction of affected lots per ADF&G Sockeye Salmon Culture Policy (SSCP). Lots that are virus-negative may be released as soon as possible. 4. IHNV (chum, Chinook, steelhead, rainbow, cutthroat): Detection of IHNV requires destruction. Operator of a facility where IHNV is detected must demonstrate remaining stocks have been isolated to prevent exposure; i.e., the facility must be minimally qualified as a partial quarantine unit (PQU). 5. ERM: Same as for adult fish except if diagnosed in the diseased state with significant mortality, destruction of the lot may be required. 6. ICH: Seawater release allowed. Freshwater, treat and release to minimize exposure of other hatchery stocks.
c. Transplant of adults, juveniles or eggs, to a watershed with other significant (resource value) stocks of salmonids.	Stocks to be transplanted: Juveniles and eggs: If no disease history then prior year samples from spawning or post-spawned adult fish recommended. Adults: If no disease history then samples of adult fish (spawning) stock to be transplanted *required* prior to transplant in year of transport. Stocks in receiving watershed: If stocks to be transplanted are negative for finfish pathogens then there is no need to sample stock for disease in the recipient watershed. If pathogens are detected in donor fish or the intent is to establish a broodstock source then prior year sampling of resident fish is strongly recommended. Sampling should include all stocks determined to be significant by area biologists. In order to develop a disease history, stocks in receiving watershed should have 60 samples collected from adult fish (spawning) for examination. If fish stocks having a known carrier state of a fish pathogen are to be transplanted and 60 resident fish are not available for examination, then the resident stocks are presumed negative for all pathogens. In any case, Class II criteria apply.

-continued-

Table 1. Page 2 of 2.

A. Wild fish transplants	Disease considerations
	Class II disease criteria: 1. BKD and Furunculosis: If stocks in receiving watershed have zero pathogen prevalence, then stock proposed for transplant must also have zero prevalence (min. sample size equals 60). The applicant is responsible for obtaining a sample of 60 adult fish. If adequate sample numbers of transplanted fish are unavailable, the transplant cannot be made. If stocks in the receiving watershed are positive for the agents of BKD or furunculosis, then the transplanted stock must not exceed levels in Schedule I. *A. salmonicida* in the receiving and donor watersheds should be confirmed by culture due to potential nonspecific fluorescence by FAT. 2. IHNV: No samples required for anadromous sockeye except to establish a disease history: all stocks are carriers. Stocks of kokanee may be negative for IHNV and must be sampled. SSCP procedures are required for spawning all sockeye salmon and kokanee. Transplant of sockeye or kokanee into nonsockeye systems having IHNV-susceptible species is discouraged. Evaluation is on a case-by-case basis regarding the resource value of the susceptible species at risk in the recipient or nearby watersheds. Transplant of IHNV-susceptible species to a watershed containing sockeye or kokanee would be evaluated on a case-by-case basis and may not be rejected on the basis of fish health concerns. Applicant and resource managers must accept the possible loss of transplanted fish or condemnation of the donor stock due to IHNV. Transplant of Chinook, chum, rainbow, steelhead, or cutthroat into a nonsockeye system from a system with sockeye will require virus sampling. Any virus-positive stock would be disqualified. If virus-negative, these species would be potential IHNV carriers, and decision criteria for sockeye and kokanee transplants would apply. 3. ICH: If there is a disease history of Ich then transplant is not permitted unless receiving waters also have a history of Ich. 4. ERM: Same as for BKD and furunculosis except if diagnosed in the diseased state with significant mortality, destruction of the lot may be required.
2. To a hatchery	
a. Quarantine Unit (QU). See Table 5.	Class II disease criteria: No constraints for pathogens in carrier state since they will be in isolation.
b. Other than a QU	Class II disease criteria: 1. If no other stocks are present at hatchery, criteria in Section A.l.a. apply. 2. If other stocks are present in the hatchery and their disease histories are negative for pathogens, then the transplanted stock history must be negative. If other stocks are present in the hatchery and they have a history of BKD, furunculosis or ERM agents then the transplanted stock must meet the criteria for Schedule I. 3. If a pathology-approved Partial Quarantine Unit (See Table 5) is to be used, then other stocks at the hatchery are not a concern. 4. In either case (except effluent depuration in a PQU), if there are wild salmonids present in the hatchery watershed criteria in A.l.c apply.
3. To a flow-through research facility/aquarium	
a. Local fish and invertebrates	No restrictions provided animals and water source are from "local" waters adjacent to the research facility or aquarium
b. Nonlocal fish and invertebrates	Effluent depuration or treatment required with no release of animals and proper disposal of dead animals by incineration or landfill.

[a] Classes I, III, and IV finfish diseases are addressed sufficiently in the regulation section.

Table 2.–Broodstock screening for egg takes.

B. Broodstock screening for Egg Takes[a]	Disease considerations
1. Egg take at hatchery (indigenous stock)	
a. For release of progeny at hatchery	Provided an acceptable disease history within the broodstock has been established and fry performance has indicated no disease concerns, no disease screening required, but recommended every other year. Disease outbreaks in juveniles or significantly high levels of a Class II pathogen in the broodstock may require corrective action and more sampling.
b. For release of progeny at another site	Samples can be taken in year prior to initial egg take. Class II disease criteria: 1. BKD: Prevalence of Rs in a brood source may require Family Tracking[b] as an acceptable control measure. 2. Furunculosis and ERM: Not considered (B.2.a) unless (1) there has been recent problems within the disease histories or (2) it is a new stock without prior disease history, in which case screening is done to establish a disease history. 3. IHNV (sockeye, kokanee): Sample size equals 60 adult (spawning) fish in prior year for establishing population prevalence. Spawned fish can be used thereafter at the egg take to determine annual IHNV risk. SSCP procedures must be used for spawning all sockeye salmon and kokanee. 4. IHNV (chum, Chinook, steelhead, rainbow, cutthroat): Screening for IHNV would not be routine in indigenous nonsockeye hatchery stocks unless IHN disease or other virus exposure is suspected. For large scale egg takes, sampling in year prior is recommended. Any detection of IHNV would require the destruction of the broodstock and any eggs spawned and condemnation of the broodstock as a future source of eggs.
2. Egg take at a site remote from hatchery	
a. For stocking of progeny back to system of origin	Class II disease criteria: 1. Approved QU, no constraints. 2. Non-QU (sampling required but recommended in year prior to egg take). 3. BKD: Rs prevalence in brood source requires Family Tracking. For hatcheries requiring reuse or recirculation of water, the consequences of introducing Rs from outside cannot be tolerated. Family Tracking must be done or a known Rs-negative stock is required. 4. Furunculosis and ERM: No specific limitation. High-risk stocks should not be used if low risk stocks are available. Egg disinfection is required; pathology staff may monitor/assist at egg takes, and may require fry samples prior to release depending upon fry performance. There is no evidence that vertical transmission of *A. salmonicida* or the ERM agent occurs WITHIN the eggs of salmonids. Consequently, eggs from a low number of carrier broodfish pose no additional risk if rigorous external disinfection is practiced. However, the risk of inadequate egg disinfection increases with increasing numbers of carrier broodfish. 5. IHNV (sockeye, kokanee): Sample size equals 60 spawning adult fish in year prior and is required for disease history information; specific precautions recommended by pathology staff will depend on facility type, location, and fish handling capabilities. All anadromous stocks of sockeye are carriers. SSCP criteria must be used for spawning all sockeye and kokanee. After establishment of a disease history, subsequent sampling may include 60 fish used in the egg take to monitor the prevalence of IHNV brought into the hatchery with gametes. 6. IHNV (chum, steelhead, rainbow, Chinook, cutthroat): In a system with sockeye, 60 samples from the desired susceptible species (spawners) are required in year prior. Any detection of IHNV in samples prohibits use of that stock for eggs. 7. ICH: Not applicable.

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B. Broodstock screening for Egg Takes[a]	Disease considerations
b. For release at the hatchery OR c. For release at a remote site	Same criteria as B.l.b. Also, IHNV-susceptible species other than sockeye salmon from sockeye systems are not recommended for use and will be considered on a case-by case basis.
d. Stock originating from hatchery fish at remote site for release into barren system or terminal seawater release site (no watersheds)	Same criteria as A.1.b and C.3.b.
e. Stock originating from hatchery fish at remote site for release to a system with salmonids.	Same criteria as A.l.c. and C.4.

[a] The following disease considerations regarding BKD are an alternative to the preferred use of broodstocks having no history of the Rs agent. Toleration of minimal levels of this disease agent in stocks used at any facility is allowed only if: an alternative stock(s) is unavailable; other circumstances specific to ongoing programs leave no practical alternative; other mitigating procedures such as Family Tracking are practiced to reduce disease risk.

[b] For small populations of less than 1,000 where a sample of 60 adult fish in one year would constitute significant loss, alternative arrangements with the pathology section may include sampling fish over a period of years prior to the proposed egg take. Under well justified circumstances an alternative is Family Tracking that requires kidney samples during the egg take. Family Tracking requires keeping egg lots separate during water hardening, disinfection, and incubation in Heath Trays until testing of individual parents is completed. Egg lots from Rs-positive parent fish are destroyed.

Table 3.–Disease history of juvenile fish prior to release.

C. Disease history of juvenile fish prior to release	Disease considerations
1. At the hatchery site	Prerelease examination of juvenile fish is not done unless mortality or other clinical signs of disease or otherwise poor performance prior to release warrant concern by the Fish Pathology Section or the broodstock disease history at egg take was positive for Rs and Family Tracking was not done, or both. Class II disease criteria: 1. BKD: If no significant mortality, no restriction. A total cumulative mortality equal to or greater than 5% in 90 days prior to release attributable to BKD will prohibit release. It is the long-range goal that all facilities meet the detection criteria in Schedule I. Those that do not but have total cumulative mortalities of less than 5% in the 90 days prior to release can release provided there will be future alterations in the physical plant or operations to achieve the limits of Schedule I within six years from date of problem occurrence, or both. 2. Furunculosis: Must be treated until mortality reaches background level (.03% per day). A withdrawal period after drug therapy may be required before release. 3. IHNV (sockeye, kokanee): Infected lots, as determined by clinical signs or detection of IHNV must be immediately destroyed per SSCP. Lots that are negative for virus may be released as soon as possible. Additional virus detection or clinical signs will require destruction of affected lots. 4. IHNV (chum, Chinook, steelhead, rainbow, cutthroat): Same as for sockeye except detection of IHNV in fry will require destruction of the inventory of that stock unless demonstrated that lots within that stock have been isolated and not exposed to the virus. It also must be demonstrated that isolation has been maintained for other susceptible stocks on site to assure they have not been exposed to IHNV. Otherwise, the destruction of the other exposed stock(s) will be required. 5. ERM: If diagnosed as clinical disease with significant mortality, elimination of a stock may be required, depending upon circumstances. 6. ICH: Treat prior to release.
2. Return to system of origin	Class II disease criteria: 1. BKD: If broodstock was Rs-negative, juveniles are assumed negative unless found to be Rs-positive by examination. In this case, release cannot occur (to the system of origin) unless the broodstock, not the hatchery water supply (such as in a QU or PQU), is determined to actually have been positive whereby release will be considered on a case-by-case basis. If the broodstock had Rs-positive samples and progeny egg lots were not culled by Family Tracking then a prerelease sample of 60 juvenile fish will be required and cannot exceed Schedule I for release authorization. 2. Furunculosis: If clinical disease is present, treat and release when mortality returns to a background level and prevalence does not exceed Schedule I. A withdrawal period after drug therapy may be required. However, if the brood source had no confirmed history of *A. salmonicida*, release of positive juveniles (to the system of origin) in the carrier state will not be authorized. 3. IHNV (sockeye, kokanee): Infected lots with clinical signs of disease or detectable virus must be destroyed per SSCP. Virus-negative lots may be released while further detections of IHNV or observed clinical signs in additional fish lots will require their destruction. 4. IHNV (chum, Chinook, steelhead, rainbow, cutthroat): Same as for sockeye except detection of IHNV in fry requires destruction of the inventory of that stock unless demonstrated that lots within that stock have been isolated and not exposed to the virus. It also must be demonstrated that isolation has been maintained to assure that other susceptible stocks on site have not been exposed to IHNV. Otherwise, the destruction of the other exposed stock(s) will be required. 5. ERM: If clinically diseased with significant mortality, elimination of a stock may be required depending upon circumstances. If detected in the carrier state and the brood source had no confirmed history of the ERM agent, release of juveniles back into the system of origin will not be authorized. 6. ICH: Seawater release allowed. Freshwater release may be allowed on a case-by-case basis after treatment to minimize exposure of other hatchery stocks.

-continued-

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C. Disease history of juvenile fish prior to release	Disease considerations
3. To barren systems (no salmonids)	
a. Closed system (landlocked lake)	A landlocked lake has no outlet with direct or indirect connection to another watershed. Class II disease criteria: 1. ERM: If detected in a carrier state, transplant would be decided on a case-by-case basis. If clinically diseased with significant mortality, destruction of the lot(s) may be required. 2. All other Class II diseases: no restriction for pathogens in carrier state. 3. Release of fish in the diseased state (excluding ERM) would be considered for research purposes only.
b. Open system	Class II disease criteria: 1. BKD: No significant mortality and immediate disease history of hatchery performance cannot exceed levels in Schedule I. 2. Furunculosis: As indicated by FAT with confirmation by isolation. If clinically diseased, treat and release when mortality becomes insignificant and prevalence does not exceed Schedule I. If prevalence of infection exceeds Schedule I, fish cannot be released. A withdrawal period after drug therapy may be required before release. 3. IHNV (sockeye, kokanee): Release if no disease. Clinical signs of IHN or detection of virus requires destruction of affected lots per SSCP. Release virus-negative lots as soon as possible. Subsequent to release, mortality or detection of virus from additional lots will require their destruction. 4. IHNV (chum, Chinook, steelhead, rainbow, cutthroat): Detection of IHNV requires destruction of that stock unless demonstrated that unaffected lots have not been exposed and that remaining stocks on site have been isolated to prevent virus exposure, i.e., the facility must qualify as a PQU. 5. ERM: Same as for C.3.a. 6. ICH: Seawater release allowed. Freshwater release, treat and release to minimize exposure of other hatchery stock.
4. To systems with other significant (resource value) stocks of salmonids	
a. Closed system (landlocked lake)	Class II disease criteria:
b. Open system	1. BKD: If Rs detected within the prior two years of stock disease history or within the present inventory of juveniles prior to release, then those juveniles cannot be released unless other species or stocks at release site or upstream in the tributary of release also have a history of Rs. In this case, the carrier state in released juveniles cannot exceed levels in Schedule I. Release is not allowed if clinically diseased as indicated by significant Rs related mortality (equal to or greater 5%) occurring within 90 days prior to release date. 2. Furunculosis: If detected in the present inventory of juveniles prior to release then those fish cannot be released unless other species or stocks at release site or upstream in the tributary of release also have histories of the causative agent. In this case released juveniles cannot exceed levels in Schedule I. If clinically diseased, fish must be treated until mortality is insignificant and carrier state does not exceed Schedule I. A withdrawal period after drug therapy may be required before release. 3. IHNV (sockeye, kokanee): Release allowed provided no clinical signs of IHN or virus is detected. Release into nonsockeye systems having IHNV-susceptible species not recommended and will be evaluated on a case-by-case basis.

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C. Disease history of juvenile fish prior to release	Disease considerations
	4. IHNV (chum, Chinook, steelhead, rainbow, cutthroat): Detection of IHNV requires destruction of affected lot(s) and entire inventory of that stock and others on site unless isolation from virus exposure can be demonstrated. Transplant of Chinook, chum, rainbow or steelhead into a nonsockeye watershed from a hatchery on a sockeye watershed will be evaluated according to sockeye transplant criteria IF such a stock has not been isolated or has been exposed to a water supply containing rearing or spawning sockeye during any part of the life cycle.
	5. ERM: Same as for furunculosis except if clinically diseased with significant mortality, destruction of the lot may be required depending upon circumstances.
	6. ICH: Seawater release allowed. Freshwater release may be allowed on a case-by-case basis after treatment to minimize exposure of other hatchery stocks
5. Remote seawater release for terminal fisheries	
	Class II disease criteria:
	1. BKD and Furunculosis: An exception to the Schedule I carrier rate criteria may be made on a case-by-case basis with large inventories of presmolts for release into a "mop up" terminal harvest fishery. Depending upon the fishery, natural stocks are exposed to negligible disease risk when hatchery returns are completely harvested. Release of smolts is not permitted when clinically diseased as indicated by a $\geq$5% cumulative mortality occurring within 90 days prior to seawater release.

Table 4.–Transfers between hatcheries.

Transfers between hatcheries	Disease considerations
1. Eggs	Class II disease criteria: 1. BKD: Transfer not allowed unless the receiving hatchery has a history of Rs in resident stocks or Family Tracking is done, or both. Eggs from Rs-positive parent fish from the donor facility are destroyed before transport or while in isolation at the receiving facility. Family Tracking may reduce or prevent amplification of the carrier rate within the broodstocks returning to both facilities. 2. Furunculosis: Eggs from high risk stocks not recommended if low risk stocks are available. However, no restrictions for criteria as previously stated (B.2.a). 3. IHNV (sockeye, kokanee): If the receiving facility is qualified to take eggs directly from a broodstock, then the same facility can receive eggs from another facility. 4. IHNV (chum, Chinook, steelhead, rainbow, cutthroat): Eggs from IHNV-susceptible species from a sockeye facility are not recommended for transfer to a nonsockeye facility unless the receiving facility is a QU or the stock has been adequately isolated and not exposed to a water supply containing rearing or spawning sockeye during any period of the life cycle. 5. ERM: Same as for furunculosis. 6. ICH: Not applicable.
2. Fish (from hatchery to hatchery, excluding a QU).	Class II disease criteria: 1. BKD: Not allowed if fish to be transferred have had BKD or if the Rs agent has been detected within the previous two years of stock disease history unless receiving facility has a history of Rs. In the latter case, the detection level in the juveniles to be transferred cannot exceed Schedule I and no significant BKD-related mortality can have occurred. 2. Furunculosis: Not allowed if fish to be transferred have had furunculosis unless receiving facility has a history of furunculosis. In the latter case, the detection level in the juveniles to be transferred cannot exceed Schedule I and no significant furunculosis-related mortality can have occurred. 3. IHNV (sockeye, kokanee): Transfer to another sockeye facility allowed unless there are clinical signs of IHN confirmed by virus isolation. Not permitted to a non-QU that contains nonsockeye susceptible species. 4. IHNV (chum, Chinook, steelhead, rainbow, cutthroat): Can be transferred from a nonsockeye facility to a sockeye facility if the latter is a QU where fish can be reared on an IHNV-free water supply and are not intended for adult return to the same site as the sockeye returns. Screening for IHNV in nonsockeye susceptible species is not necessary when from nonsockeye water supplies unless there is clinical disease suggestive of IHN. Clinical disease with isolation of IHNV will result in the destruction of any fish stocks. IHNV-susceptible stocks cannot be transferred from a non-QU sockeye facility to a nonsockeye facility having other susceptible species or stocks unless the receiving facility is also a QU. 5. ERM: Same as for furunculosis except diseased fish sustaining significant mortality may have to be destroyed depending upon circumstances. 6. ICH: Not allowed if the fish to be transferred have had an outbreak of Ich unless the receiving facility also has a history of Ich in its water supply. In the latter case, the fish for transfer must not be sustaining significant mortalities, otherwise treatment and holding of fish will be necessary at the donor facility until mortalities fall within background levels.

SOCKEYE SALMON CULTURE POLICY (SSCP)

ISSUE

Artificial propagation of sockeye salmon can be seriously limited by IHNV occurring naturally in all anadromous sockeye salmon stocks in Alaska. The disease has caused catastrophic mortality of juvenile sockeye salmon in hatcheries throughout Alaska. The causative agent is a *Novirhabdovirus* that has evolved into three geographic genotypes among which the U clade has adapted to sockeye salmon in Alaska and British Columbia (Emmenegger et al. 2000). The other genotypes, M and L, have adapted to and infect other salmonid species causing serious mortality of Chinook salmon, Atlantic salmon and rainbow/steelhead trout in the Pacific Northwest including Idaho (Kurath et al. 2003). Stringent control methods are required in Alaska to help prevent the potential for the U clade virus to infect and adapt to other IHNV-susceptible salmonid species.

POLICY

Following the 1980 IHN epizootics, the most logical disease control concepts and techniques applicable to sockeye salmon culture were assembled into an ADF&G SSCP.[1] This policy has undergone some revision since its origin but the key criteria remain unchanged. These criteria include 1) virus-free water supply, 2) rigorous disinfection procedures, 3) compartmentalization of eggs and fry during incubation and rearing, and 4) immediate destruction of fish infected with IHNV followed by disinfection to contain the spread of the virus within the hatchery and prevent exposure of wild fish stocks. Additional rationale and procedures for avoiding IHNV in sockeye culture are published under separate cover in the *Alaska Sockeye Culture Manual* (McDaniel et al. 1994).

SHELLFISH CULTURE

Importation of live shellfish species into Alaska for mariculture purposes

{Article 3, 5 AAC 41.070 (b)(d)}

Pacific oyster (*Crassostrea gigas*)

Spat (seed) less than or equal (≤) to 20 mm shell diameter are permitted for importation only from ADF&G-certified sources on the Pacific coast of North America and Hawaii.

Weathervane scallops (*Patinopectin caurinus*)

Weathervane scallops originating from wild stocks or cultured stocks in the Southeastern Alaska and Yakutat areas may be imported and released only into the same area waters and only from ADF&G certified sources.

Certification requirements for shellfish importation

1. Separate broodstocks must be from the same sources and locations from year to year (stock for certification cannot be composed of multiple stocks from different locations) and must be physically or geographically isolated from noncertified stocks during all stages of culture.

[1] These guidelines were developed by staff within the ADF&G Fisheries Rehabilitation, Enhancement, and Development Division and included R. Burkett (chair), R. Saft, J. Burke, J. Sullivan and B. Kepshire.

2. There must be no detection of disease or pathogens of transport significance in the stock to be imported. *Mytilicola* sp. is indigenous to Pacific oyster stocks in the Pacific Northwest and requires a maximum size limitation of spat (≤20 mm) to reduce the likelihood of successful establishment of the parasite in Alaskan shellfish.
3. There must be no detection of disease or pathogens of transport significance in other shellfish species and stocks from the certified facility or in the hatchery water supply. Depuration of the water supply to standards established by the ADF&G Fish Pathology Section may be required.
4. The seed stock proposed for certification must be physically or geographically isolated from noncertified stocks through all stages of culture.
5. There must be a written proposal with an operational plan providing details of the isolation facility, seawater source and procedures of physical separation of the stock identified for certification.
6. There must be a schematic layout of the facility and a map locating the facility, broodstock location, and any nearby hatcheries or shellfish beds.
7. Disease history information must be provided for all stocks and species of shellfish on site based on past production experience and laboratory reports from previous pathology examinations.
8. Samples for histological examination must be submitted to a laboratory approved by the ADF&G Fish Pathology Section. This must be done at least 60 days prior to transport permit application and approval to allow processing and pathology examination.
    - 60 adult animals from the parent broodstock
    - 60 spat from the cohort of animals proposed for import
    - 1,000 larvae (if applicable)
    - PCR testing of adult and juvenile oyster for *Mikrocytos mackini* and/or *Haplosporidium* sp. may be required as part of the certification
9. Renewal of certification is done annually and requires histological examination of 60 spat from the year class to be imported accompanied by an updated disease history for all stocks, species and life stages on site and a statement of hatchery performance reviewing success, problems, mortality, etc during the previous growing season. Certification is generally valid for a year from the date of sample collection for pathology examination.
10. Certification will become invalid if a Class I pathogen or other pathogen causing significant disease or mortality is detected at the facility, or an uncertified shellfish stock is brought into the rearing or grow-out areas utilized for the certified stock.
11. All lots of imported spat must be free of pests and other nontarget species. This can be accomplished by immersion of spat in a freshwater solution containing 10 ppm of chlorine bleach for 15 min.
12. Each Alaskan buyer must have a valid shellfish transport permit issued by the ADF&G mariculture coordinator and a copy of the permit must be on file at the certified facility before shipment of oyster spat into Alaska.

13. Certification applications, certification renewals and other required information are submitted to the Mariculture Coordinator, ADF&G, Commercial Fisheries Division, P.O. Box 115526, Juneau, AK 99811-5526.

Requirements for transport of live oysters and other indigenous shellfish species within Alaska for mariculture purposes

1. An approved shellfish transport permit is required.
2. An acceptable disease history is required for the shellfish stock to be transported, *from the donor site,* regardless of whether the stock originated from a certified source or whether a disease history exists for the stock at another site. Disease histories are site and stock specific.
3. If no disease history is on record, then at least 30 live animals must be submitted to the ADF&G fish pathology labs for histological examination at least 60 days prior to issuance of a shellfish transport permit.

Grow-out sites

If only juveniles are present at a grow-out site and are to be transported, then juvenile animals are submitted for examination.

When adults and juveniles are present on the same site, the following apply.

1. If animals are to be transported to establish broodstock elsewhere, then adult animals are required for examination.
2. If juveniles are to be transported to multiple sites for grow-out and market, then adults are required for samples. If this continues on a regular basis then the site will qualify as a seed distribution facility that may or may not require annual juvenile examination depending on the acceptability of the disease history.
3. Transport of native shellfish species collected on culture gear requires juveniles for samples unless 30 adult animals are available.

Hatcheries and seed distribution facilities

1. Shellfish hatcheries require annual or every-other-year inspections of the hatchery facility and complete disease histories on all adult broodstocks. Yearly histological examinations of juveniles from each stock that are shipped to various grow-out sites may or may not be required depending on the acceptability of the disease histories.
2. Seed distribution facilities having no adult animals on site may or may not require annual histological examination of juveniles shipped to various grow-out sites depending on acceptability of the disease histories.
3. The definition of how far transport must be to require pathology examination is defined by the discreetness of stocks or populations with regard to the planktonic drift zone (RaLonde 1993) of larval dispersal by ocean currents, etc. If this cannot be resolved to the satisfaction of the department, any movement, regardless of distance, will require submission of samples for histological evaluation.

4. Shellfish samples for histological examination will be required from any grow-out site, shellfish hatchery, or seed distribution facility, when there is unusual shellfish mortality exceeding the expected background levels of if clinically abnormal animals are observed.
5. Detection of any Class I disease agents exotic to North America will require quarantine, stoppage of effluent discharge, complete destruction of affected shellfish stocks with proper disposal and disinfection of the facility. Detection of Class I pathogens exotic to Alaska, but not North America, may require all of the above if the agent poses a threat to wild or hatchery shellfish stocks.

Requirements for export of live shellfish outside Alaska

1. An approved shellfish transport permit is required.
2. Authorization from receiving state authorities is required prior to issuance of an approved ADF&G shellfish transport permit.
3. The Fish Pathology Section will provide a disease history for the stock if one is on record but will not certify a stock as *disease free* and is not obligated to provide additional pathology examination should that be required by the receiving state authorities.

GENERAL GUIDELINES AND RESPONSIBILITIES OF THE ADF&G FISH PATHOLOGY SECTION

MISSION STATEMENT

The Fish Pathology Section monitors and controls finfish and shellfish diseases statewide (according to Title 16 of the Alaska Statutes) by oversight of wild and hatchery fish and shellfish health, conducting diagnostic surveys, developing finfish and shellfish disease policies and by advising the commissioner of ADF&G and other state and federal authorities on fish disease issues.

FACILITIES

There are two state-of-the-art laboratories fully equipped for complete diagnostic capabilities encompassing the disciplines of bacteriology, virology, serology, histology, DNA probe, PCR, immunocytochemical staining and transmission electron microscopy.

Anchorage diagnostic laboratory: This laboratory is located in Region II to adequately service approximately 44% (14) of the Alaskan hatchery facilities and other user groups located in the Anchorage, Kodiak, Cook Inlet, Kenai Peninsula, Prince William Sound and Fairbanks areas. The Anchorage laboratory also has a small wet lab space to hold live fish for disease transmission studies and performs the PCR work.

Juneau diagnostic laboratory: This laboratory is strategically located in Region I to adequately service the other existing 56% (18) of the Alaskan hatcheries and other user groups located in the Southeastern panhandle including the Juneau, Petersburg, Wrangell, Ketchikan and Baranof Island areas. The Juneau lab has additional capabilities for the enzyme-linked immunosorbent assay (ELISA) and transmission electron microscopy.

STAFF

Most fish pathology staff members have several years of experience in the fish health or medical technology disciplines. Collectively, professional degrees and staff training are in the fields of

microbiology, virology, finfish/shellfish pathology and veterinary medicine. There are currently five full-time staff positions.

Anchorage diagnostic laboratory: Staff consist of a Laboratory Technician, a Microbiologist II and a Fish Pathologist in charge of day-to-day functions.

Juneau diagnostic laboratory: Staff consist of a Microbiologist I and the Principal Fish Pathologist/Fishery Scientist I who administers the fish pathology program statewide and supervises both laboratories.

PROGRAM RESPONSIBILITIES

Diagnostic services. The fish pathology laboratories provide complete diagnostic services according to Bluebook standards of the Fish Health Section of the American Fisheries Society to all user groups statewide for examination of wild and hatchery finfish and shellfish. The caseloads for both laboratories are generated by the following user needs or duty requirements.

1. Services provided for fish health problems occurring at approximately 32 fish-rearing facilities statewide.
2. The laboratories address wild fish or shellfish health problems reported by agencies or private user groups. Notable examples include viral hemorrhagic septicemia virus (VHSV) in Prince William Sound herring, Bitter Crab Syndrome in Tanner crabs, fish kills, fish abnormalities or product quality control problems discovered by fish processors, commercial or sport fisherpersons.
3. Finfish or shellfish transport permits for instate movement each require establishment of a disease history. Many permitted shellfish farms move or sell shellfish across the state requiring pathology examination by ADF&G.
4. Occasionally out-of-state agencies or laboratories request fish or shellfish diagnostic services. For example, Alaska is a cooperator in the National Wild Fish Health Survey conducted by the U.S. Fish and Wildlife Service.

Caseload effort. In FY 2013 the fish pathology labs processed 143 accession cases with a total of 9,912 animals examined requiring a total of 19,033 diagnostic tests. Each case accession number requires a written laboratory report issued to the client submitting the samples and appropriate distribution of copies. This FY 2013 effort compares to a 10-year annual average of 133 cases, 9,930 animals examined and 14,646 tests performed.

2013 Percentile of Testing Effort by User Group

1. State Sport Fish = 18.9%
2. Other State = 4.9%
3. Private Nonprofit = 59.4%
4. Federal = 6.3%
5. Sci-Ed = 2.8%
6. Miscellaneous = 7.7%

Hatchery support. The Fish Pathology Section provides a wide range of fish health services.

1. The staff in both laboratories provide advice and supervision for fish health activities at 32 fish-rearing facilities statewide.

2. Onsite fish health and physical plant hatchery inspections are provided along with advice on proper sanitation procedures. Each hatchery inspection requires a written report issued to the hatchery manager and appropriate distribution of copies.
3. Staff provide diagnostic services, recommendations for appropriate preventative measures, and therapy to control fish disease problems.
4. Staff assist hatchery personnel with collection of disease samples when appropriate.
5. Staff conduct fish health workshops in finfish and shellfish disease recognition to train Alaskan hatchery personnel. Generally 25–30 students attend representing most hatchery facilities statewide. Lectures, notebooks and laboratory training are provided.

   Staff at several remote hatcheries have used fish pathology workshop training to develop a health condition profile following the Goede (1997) method where fish are periodically examined for general appearance and disease conditions before serious mortality can occur. Some of these facilities have necropsy areas where basic microscopy and bacteriology are performed to make preliminary descriptive observations of any problem prior to consulting with fish pathology staff. The turn-around time for a diagnosis in such cases has been much reduced because of this training.

Finfish and shellfish disease management through regulatory authority. The ADF&G Fish Pathology Section has regulatory responsibilities as outlined in Title 16 of the Alaska Statutes. Specifically these duties fall within these general categories listed.

1. Review of all transport and fishery resource permits for instate movement and export of finfish, invertebrates and aquatic plants to evaluate health concerns that could occur due to animal or plant movement (5AAC 41.005, Permit Required; 5AAC 41.030, Permit Issuance or Denial; 5AAC 41.050, Permit Conditions).

   In 2013 Pathology staff reviewed approximately 200 permit applications for fish and shellfish transport or possession.

2. Develop and maintain a current finfish and invertebrate statewide disease history data base for the purpose of evaluating finfish or shellfish transport permits and use in other policy decisions (5AAC 41.020, Inspection for Disease of Brood Stock; 5AAC 41.080, Reporting and Control of Fish Diseases at Egg-Take Sites, Hatcheries and Rearing Facilities).

   The Fish Pathology Section maintains an extensive aquatic animal disease history data base that extends back to the late 1970s.

3. Oversight and periodic inspection of hatcheries (5AAC 41.080, Reporting and Control of Fish Diseases at Egg-Take sites, Hatcheries and Rearing Facilities; AS 16.05.868, Certified fish health specialist by the Fish Health Section of the American Fisheries Society) to advise, prevent and control fish diseases in hatcheries and to prevent pathogen exposure of wild fish stocks.

   In conjunction with this responsibility the statewide ADF&G fish disease policy was established in 1987 to govern day-to-day fish health concerns, assist pathology review of transport permits and provide additional guidelines on shellfish health. Proposed changes in state regulations to accommodate aspects of this policy were approved by the Board of Fish and codified in 2011. A separate document, *Alaskan Sockeye Salmon Culture Manual*,

ADF&G Special Publication No. 6 (McDaniel et al. 1994) provides details of the ADF&G sockeye salmon culture policy to control IHNV that was first implemented in 1981.

4. Advise departmental staff and other user groups regarding compliance with the current ADF&G fish disease policy when developing hatchery annual management plans, stock management plans, fish transplant strategies and policies, or other enhancement projects to establish populations of fish.
5. Disease certification of Pacific oyster seed for import into Alaska from the Pacific Northwest and Hawaii, weathervane scallop seed from stock originating from Southeast Alaska and Yakutat (5AAC 41.070, Prohibitions on Importation and Release of Live Fish). These are the only aquatic animal species allowed for import into Alaska except for ornamental fish not reared for human consumption or released into state waters.
6. Require the destruction of diseased fish when mandated by the severity of the pathogen as determined by 5AAC 41.080 (Reporting and Control of Fish Diseases at Egg-take Sites, Hatcheries, and Rearing Facilities) and by the current ADF&G Fish Disease Policy.

Research. The Fish Pathology Section conducts applied research to achieve these objectives.

1. Disease transmission studies using onsite wet lab facilities to determine pathogenicity and mode of transmission of new or poorly described disease agents (VHSV, *Phoma*)
2. Morphological description and biochemical characterization of new or poorly described disease agents (Bitter Crab Syndrome, VHSV, *Phoma*)
3. Evaluation of new techniques for the detection of finfish and shellfish disease organisms (ELISA, Dot Blot, DNA probe, PCR, QPCR)
4. Distribution surveys of specific disease agents in finfish and shellfish stocks statewide (IHNV, VHSV, Bitter Crab Syndrome, Viral Erythrocytic Necrosis Virus, BKD, *Ichthyophonus*, Infectious Salmon Anemia Virus)
5. Maintain a current knowledge of existing and emerging research findings on new finfish and shellfish diseases and diagnostic methods through review of scientific literature, professional development training and attendance of professional meetings and workshops

   Additionally, two staff of the Fish Pathology Section received Fisheries Rehabilitation, Enhancement and Development division awards for technical achievement in 1988 and 1990 for the discovery and characterization of VHS virus in Alaska and for the development of the ELISA to screen Alaskan fish for the BKD agent.

Public education. As part of ADF&G, the Fish Pathology Section provides information to the public regarding finfish and shellfish disease issues and inquiries. This has been accomplished in the following ways.

1. Staff provide one-on-one information to fisherpersons, processors, other government agencies and the media regarding finfish and shellfish abnormalities, mortality, etc. by telephone, email, laboratory reports and scheduled meetings.
2. Staff conduct informational laboratory tours to elementary through college-level student groups.
3. Staff support local schools with mentoring of students for science fair projects.

4. Staff publish research results in peer-reviewed journals to disseminate new information on finfish and shellfish diseases (see Fish Pathology Section Publications).
5. Staff distribute among resource agencies and user groups an informational color brochure and have a web site describing the ADF&G Fish Pathology Section program.
6. Staff distribute the *Fish Pathology Section Laboratory Procedures Manual* and descriptive field guide books on diseases of Alaskan finfish and shellfish to state hatcheries and to several fish pathology laboratories nationwide that have requested the documents as references. These references are also available on the ADF&G website. The ADF&G procedures manual was used 1) as a template for the U.S. Fish and Wildlife Service's procedures manual developed for their nine fish health centers, to implement the National Wild Fish Health Survey (True 2000), and 2) for manuals to be developed by the Oxford Cooperative Laboratory in Maryland and the Washington Department of Fisheries and Wildlife.
7. The Principal Fish Pathologist serves as finfish/shellfish disease technical representative/expert for the State of Alaska for participation in fish health issues with other state and federal agencies inside and outside of Alaska.

Affiliations with other fish health laboratories, agencies and organizations. Over several years the Fish Pathology staff have networked with other fish health laboratories outside of Alaska in various government agencies and organizations through different activities including co-authored publications in peer-reviewed journals (see publications list in Appendix B), professional committees and societies and the rare need for specialized diagnostic tests that are not routine or practical for the ADF&G laboratories. The ADF&G Fish Pathology Program is well recognized by the fish health profession within and outside of the United States.

Professional activities outside ADF&G. The Fish Pathology Section staff are involved in the following outside activities.

1. The Principal Fish Pathologist serves as the Alaska technical representative on the Pacific Northwest Fish Health Protection Committee and chaired the committee from 1994 to 1995.
2. Two staff are certified as Fish Pathologists by the Fish Health Section of the American Fisheries Society and belong to additional societies including the European Association of Fish Pathologists, the Society for Invertebrate Pathology and the American Society of Parasitologists.
3. The Principal Fish Pathologist served as president of the American Fisheries Society/Fish Health Section from 1994 to 1995 and from 2007 to 2008.
4. The Fish Pathology Section hosted two Pacific Northwest Fish Health Protection Committee meetings in Juneau (1995, 1997), the 1997 National Meeting of the Fish Health Section in Juneau attended by 80 fish pathologists nationwide including Spain and Portugal, and the Western Fish Health Workshop in 2004.
5. The Principal Fish Pathologist served as a technical advisor on fish health in Alaska for the National Marine Fisheries Service offices in Silver Springs, MD and Washington D.C. regarding the previous Australian and New Zealand trade embargos on fresh/frozen U.S. and Canadian salmon products.

6. Staff review about 10 to 15 manuscripts per year for peer-reviewed journals or proposals for outside funding from U.S. Department of Agriculture, Saltonstall-Kennedy (NOAA), various state Sea Grant programs, and the Great Lakes Fishery Trust, among others.
7. Staff attend three or four out-of-state fish health meetings annually to present research and remain current with new discoveries and technological advancements in the fields of fish and shellfish health.
8. The Principal Fish Pathologist served as a technical advisor invited by the NMFS and Idaho Fish and Game for determination of disease screening protocols for the endangered Red Fish Lake sockeye salmon program.
9. The Principal Fish Pathologist served as a technical advisor for the U.S. Fish and Wildlife Service planning of the National Wild Fish Health Survey and both labs worked cooperatively with the Service by examining large numbers of Alaskan samples.

QUARANTINE UNIT FISH HATCHERIES

Introduction. Hatcheries often support projects that require transport of wild fish or gametes from remote sites to the hatchery. Any movement of fish between areas raises concern that pathogens may be introduced. Consequently, such risk requires that measures be taken to minimize the inadvertent dissemination of diseases.

Disease screening and disinfection play major roles in reducing the risk of spreading fish pathogens. However, testing is usually limited to a few disease agents of highest concern and testing may fail to detect low carrier-state levels of a pathogen. To provide additional protection for other hatchery stocks, the operational plan for the hatchery may be required to provide isolation of a remote stock from others in the facility during incubation and rearing. Varying levels of isolation can be achieved through use of physical barriers and other safeguards in the hatchery's design. Isolation capability falls into three categories ranging from almost none to quarantine levels. However, no design is failsafe; the efficacy is determined by the operating procedures and the commitment of hatchery personnel to carry out these procedures.

Definitions. Three levels of isolation are described based on the efficacy of the hatchery design to provide barriers against the transfer of pathogens within the hatchery and to local wild stocks beyond facility perimeters. The most effective design is the Quarantine Unit (QU) that provides strict isolation. The second design has significant safeguards and is called a Partial Quarantine Unit (PQU). Table 5 outlines the differences between the QU and the PQU. Those hatcheries that cannot meet the criteria of the two isolation units fall into the third category of conventional hatchery. If disease appears in any stock within a conventional hatchery, all stocks are at a higher risk of being exposed than if they were in a quarantine unit.

Pathology guidelines recommend the development of quarantine units in hatcheries that use remote fish stocks. If disease occurs in a facility without quarantine capability, fish releases may not be authorized. At the very least, extensive testing and waiting periods may be required before fish can be certified for release. Development of quarantine facilities is an important investment for controlling pathogen spread, especially when wild fish stocks are at risk.

Table 5.– Quarantine Unit (QU) and Partial Quarantine Unit (PQU).

	Quarantine Unit	Partial Quarantine Unit
Water Source	Well, spring, or depurated having no Class I or II pathogens.	No Class I or II pathogens detected in water source, not accessible to anadromous fish; i.e., barriered lakes or streams.
Isolation Measures	Stocks separated by physical barrier during incubation.	No physical separation of stocks by a barrier during incubation.
	No water transfer between stocks during incubation or rearing.	No water transfer between stocks during incubation or rearing.
	Rearing units will be in separate rooms for each stock.	Physical separation between rearing units.
	Thorough disinfection of unit and its equipment prior to introduction of new stock.	Thorough disinfection of unit and its equipment prior to introduction of new stock.
	Separate footwear and outerwear to be left in each isolation unit/rearing room, Footbaths used when necessary.	Disinfection of footwear using footbaths upon entering and exiting isolation unit.
Effluent	Depuration.	Depuration may or may not be required.
Equipment	Separate for each incubation and rearing unit.	Separate for each incubation and rearing unit.

Classification. Hatcheries with offsite projects will be classified according to the level of quarantine criteria that have been satisfied. An ADF&G fish pathologist will determine the facility's classification based on an onsite inspection. The Fish Pathology Section recommends either ultraviolet or chlorination–dechlorination systems for effluent depuration. Regarding a worst case scenario of high flows and excess particulates, ultraviolet units must have a minimum rating of 175,000 microwatt seconds/cm^2 after 7,500 hours of lamp operation to achieve a 99.9% reduction of the more resistant fish pathogens. Any chlorination system must deliver at least a 2 ppm residual level of chlorine with a 5 minute contact time before beginning dechlorination with sodium thiosulfate or sulfur dioxide gas. The hatchery operator is responsible for ensuring that procedures necessary for quarantine culture are followed. Failure to do so will result in reclassification of the facility.

DRUGS AND OTHER CHEMICALS USED IN AQUACULTURE

Regulation of drugs and chemicals used in aquaculture

Chemicals and therapeutic drugs are used in aquaculture to improve water quality, remove or control aquatic algae or vegetation, eradicate nuisance fish species or aquatic invertebrates, immobilize fish (anesthetics), prevent infectious diseases, and to control fish pathogens when disease occurs. Disinfectants are chemicals that destroy a pathogen by contact on an inanimate surface or in ambient water. If a disinfectant is placed into the water for the purpose of treating the external surfaces of fish then it is classified as a drug. Hence, a drug is defined as any article intended for diagnosis, cure, mitigation, treatment, or prevention of disease in man or other animals, and articles (other than food) intended to affect the structure or any function of the body of man or other animals (Stefan 1992). More familiar categories of drugs used in aquaculture are vaccines to immunize fish against diseases by oral, immersion, or injection routes and antibiotics administered internally to fish either by diet or by injection. Antibacterial efficacy is usually

accomplished by disruption of bacterial metabolism, such as cell wall synthesis. A few antibiotics can be effective when used as a bath for external infections but this application for most is not cost effective or efficacious due to the high fish densities and water volumes. In aquaculture, when the use of any chemical or compound may directly or indirectly affect human health and safety—such as an animal drug, a feed additive or as a veterinary device—then it is regulated by the Federal Food, Drug and Cosmetic Act enforced by the Food and Drug Administration (FDA). Drug or chemical uses that may also affect animal safety or the environment are further regulated by the federal Environmental Protection Agency (EPA) as well as the Alaska State Department of Environmental Conservation. In some cases a compound may be regulated by all three agencies.

All life stages of salmonid fishes are considered by the FDA to be *food fish* for potential human consumption, hence, chemical/drug use in salmonid aquaculture is regulated by that agency. Concerns by the FDA over food safety, human health and environmental impacts have resulted in increasingly strict interpretation and enforcement of existing regulations. The FDA has reconsidered and rescinded previous rulings that allowed the use of many common chemicals or drugs for fisheries management.

Investigational new animal drug (INAD). These are permits for experimental use of compounds under consideration by the FDA. In the 1980s until the early 21st century, registration of previously used drugs or R&D of new drugs and chemicals for aquaculture were not of high priority for the drug/chemical manufacturers because of their limited market demand and value. Current costs for developing aquaculture drugs are difficult to substantiate and may be somewhat less than drugs for other animal industries. In 2003, development (if cost of failed drugs is taken into account) of a successful new drug in other industries was estimated to be about $1 to $1.7 billion and took about 12 years. Clinical trials required for a new label registration of an existing drug cost less at $60 to $100 million (DiMasi et al. 2003). Although aquaculture pharmaceuticals were a smaller niche market worldwide, aquaculture has grown globally and will continue to grow where development of new or existing drugs may become more cost-effective for larger drug companies. The total U.S. aquaculture production (freshwater and marine) in 2007 was valued at only $1.4 billion compared to $153.6 billion sales for all livestock, poultry, and their products in the United States. However, global aquaculture production has reached $100 billion which begins to offer drug companies a more lucrative target animal industry.

Two INAD permits have been granted to the University of Idaho for data collection mandatory for the registration of injectable (#6340) or dietary (#6013) administered erythromycin for control of Bacterial Kidney Disease (BKD). However, the injectable INAD has become inactive and now accommodated by the extra-label option for the drug which has been in short supply. Salmonid hatcheries in the Pacific Northwest and Alaska can participate on the dietary permit provided the proper paperwork and data reporting are completed and on file with the University of Idaho coordinators. The FDA still considers malachite green to be of high enforcement priority as are the following forbidden drugs: chloramphenicol, nitrofurans, fluoroquinolones, quinolones and steroid hormones. The U.S. Fish and Wildlife Service also administers INAD permits for several other candidate compounds for potential drug approval (except erythromycin) by application through the Bozeman office in Montana. The Service also is the official clearing house for information on the use of drugs in aquaculture that can be accessed at their website http://www.fws.gov/fisheries/aadap/home.htm.

Approved drugs. Currently, there are six compounds approved for treating salmonid diseases (Florfenicol [Aquaflor], Oxytetracycline Dihydrate [Terramycin 200], Sulfadimethoxine & Ormetoprim [Romet 30], Hydrogen Peroxide [Perox-Aide], Formalin [various trade names]), Chloramine-T (Halamid Aqua) and one anesthetic, Tricaine Methanesulfonate (MS-222). Sulfamerazine, although registered, is not currently marketed by its sponsor and is unavailable. The spawning aid Chorulon (chorionic gonadotropin) is also approved but requires a prescription by a licensed veterinarian. Registered compounds still have species, pathogen or environmental restrictions and withdrawal times according to their registered labels that limit their use.

Extra-label or off-label drug use (ELDU). Certain drugs approved by the FDA's Center for Veterinary Medicine for other animals or other conditions of use (i.e., treatment claims) may, under very specific circumstances, be legally used on aquatic species for which the drugs are not approved. Any such use is referred to as ELDU.

All of the following general conditions must be true before extra-label drug use (ELDU) is permissible.

- ELDU may only be prescribed by a licensed veterinarian.
- The prescribing veterinarian must have established a valid veterinarian/client/patient relationship as it relates to the specific situation under which the ELDU is being prescribed.
- Under most circumstances, ELDU does not apply to medicated feed.
- The drug being extra-labeled must be an FDA-approved drug.
- There can be no other FDA-approved drug for the particular species and condition of use for which the ELDU is being prescribed. However, there is one exception. If there is an approved drug for the species and condition of use, *but* that particular drug is ineffective for that species/condition, then another drug may be extra-labeled.
- ELDU is only applicable to therapeutic claims; i.e., a production drug such as a spawning hormone could not be extra-labeled.
- ELDU does not apply to Veterinary Feed Directive (VFD) drugs.

Veterinary feed directive (VFD). Florfenicol (Aquaflor®**)** is an example of a VFD drug intended for use in animal feeds. The use of VFD drugs is permitted only under the professional supervision of a licensed veterinarian. VFD drugs cannot be used under extra-label drug-use provisions. Prescription only drugs include the spawning inducer chorionic gonadotropin (Chorulon®) that may be used only by, or on the order of, a licensed veterinarian.

Low regulatory priority (LRP). Additional compounds have been classified as LRP that do not currently require an INAD. These compounds include:

- Acetic acid as a parasiticide for fish
- Calcium chloride to ensure proper egg hardening
- Calcium oxide as an external protozoacide
- Carbon dioxide gas as an anesthetic
- Fuller's earth to reduce the adhesiveness of fish eggs
- Garlic for control of parasitic infestations of marine salmonids

- Ice to reduce metabolic rate of fish during transport
- Magnesium sulfate to treat external parasitic infestations in fish at all life stages
- Onions to treat external parasitic infections of salmonids at all life stages
- Papain to remove gelatinous matrix of fish egg masses
- Potassium chloride as an aid in osmoregulation
- Povidone iodine as an egg surface disinfectant during and after water hardening
- Sodium bicarbonate to anesthetize fish
- Sodium chloride as an osmoregulatory aid and as a parasiticide
- Sodium sulfite to treat eggs in order to improve their hatchability
- Thiamine hydrochloride to prevent or treat thiamine deficiency in salmonids
- Urea and tannic acid to denature the adhesive component of fish eggs.

Low regulatory priority means Center for Veterinary Medicine is unlikely to object to the use of LRP substances if all of the following five conditions are met.

1. The substances are used for the listed indications.
2. The substances are used at the prescribed levels.
3. The substances are used according to good management practices.
4. The product is of an appropriate grade for use in food animals.
5. There is not likely to be an adverse effect on the environment.

Substances generally recognized as safe. The FDA has published in the Code of Federal Regulations (Title 21, Parts 182 and Part 582) an exceptionally long list of substances that are generally recognized as safe (GRAS) for their specific uses. For example, the FDA regards such common food ingredients as salt, pepper, sugar, vinegar, baking powder, and monosodium glutamate as safe for their intended use. As additional conditions for use of such substances, the FDA considers these to be GRAS substances only if they are made and used in accordance with good manufacturing or feeding practice, respectively.

Although this specific section (of the Code of Federal Regulations) refers to *substances* as opposed to *drugs*, any one of these listed GRAS substances could be defined (by Center for Veterinary Medicine) as a drug based on the intended use of the substance. If the intended use of the substance is other than that listed in 21 CFR 182 or 21 CFR 582, it is no longer GRAS, and if it were to be used in a manner consistent with FDA's definition of a drug, it would then be considered an unapproved drug and illegal to use. For example, eugenol, the primary ingredient of clove oil, is considered to be GRAS when used as a flavoring substance and adjuvant (21 CFR 582.60). However, clove oil, when used as an aesthetic on fish, is an unapproved drug and illegal to use. A complete listing of all substances defined as GRAS by FDA can be found in both 21 CFR 182 and 21 CFR 582.

Available drugs and restrictions on their use in aquaculture are constantly changing. For up to date information on all categories of drugs used in aquaculture the reader is directed to the U.S. Fish and Wildlife Service Aquatic Animal Approval Partnership Program website at http://www.fws.gov/fisheries/aadap/home.htm. The website also offers a Quick Reference Guide as well as posters for Approved Drugs and Approved Vaccines in Aquaculture that provide dosages, withdrawal times and other label restrictions on their use.

Description of FDA-approved drugs for treating diseases of food fish (including selected INAD-permitted use)

Current FDA-approved drugs commonly used in Alaskan salmonid hatcheries include the following compounds. Although the legal suppliers for fisheries use have been provided, changes may occur periodically and must be investigated by potential users. Medicated feeds are obtained from approved suppliers. Candidate drugs for approval require an INAD permit and extra-label use of approved drugs requires a prescription from a licensed veterinarian.

1. Florfenicol (Aquaflor®, Intervet/Schering-Plough Animal Health Corp) is a VFD drug approved for dietary treatment in flow-through and recirculating systems to control salmonid mortality from furunculosis caused by *Aeromonas salmonicida* and coldwater disease caused by *F. psychrophilum*. The treatment regime is 10–15 mg florfenicol per kg of fish per day for 10 days with a 15-day withdrawal time. The drug is also approved for treating catfish mortality from enteric septicemia caused by *Edwardsiella ictaluri* and from columnaris disease caused by *Flavobacterium columnare* . The treatment regime is 10–15 mg florfenicol per kg of fish per day for 10 days with a 12-day withdrawal time. The drug may also be used at a dose of 15 mg per kg of fish per day to treat freshwater-reared warmwater fish to control mortality caused by streptococcal septicemia associated with *Streptococcus iniae*.

2. a) Oxytetracycline Dihydrate (OTC, Terramycin 200, Phibro Animal Health) is an antibiotic approved for the dietary treatment of furunculosis (*Aeromonas salmonicida*), bacterial hemorrhagic septicemias from motile Aeromonas (*A. liquifaciens*) and Pseudomonas (*Pseudomonas* sp.) and infections by *Hemophilus piscium* in salmonids using dosages of 2.5–3.75 g drug per 100 lbs of fish per day for 10 days. There are no temperature restrictions and there is a withdrawal period of 21 days prior to slaughter or release. The drug is also approved for dietary use in all freshwater reared salmonids to control mortality from cold-water disease caused by *Flavobacterium psychrophilum* at a dose of 3.75 g drug per 100 lb fish per day for 10 days with the same withdrawal time and no temperature restrictions, and for all freshwater-reared *Oncorhynchus mykiss* to control mortality from columnaris disease (*F. columnare*) under the same treatment regime as above.

   Terramycin 200 is also approved for catfish to control bacterial hemorrhagic septicemia (*A. liquefaciens*) and Pseudomonas disease (*Pseudomonas* spp.) using 2.5–3.75 g drug per 100 lb fish per day for 10 days with a 21-day withdrawal time. Treatment is restricted to water temperatures at or above 62°F (16.7°C).

   Lobsters are also included on the label for dietary treatment of gaffkemia (*Aerococcus viridans*) at a dose of 1 g per lb of medicated feed per day for 5 days with a withdrawal period of 30 days. The drug may be in the feed as the sole ration.

   Other approved uses of the drug include marking skeletal tissue in Pacific salmon using 250 mg drug per kg fish per day for 4 days. The drug can be in the feed as the sole ration and salmon must be <30 g in size and require a 7-day withdrawal time.

   b) Oxytetracycline Hydrochloride is available in various forms (Oxytetracycline HCl Soluble Powder, 343 IVX Animal Health; Terramycin 343, Aquatic Health Resources; Tetroxy® Aquatic Soluble Powder, Bimeda) for skeletal marking achieved by immersion of finfish fry and fingerlings in 200–700 mg of the buffered drug per L of water for 2–6 hr.

Terramycin has also been effective and widely used for treatment of vibriosis (*Vibrio anguillarum*) and enteric redmouth (ERM, *Yersinia ruckeri*) and has shown some efficacy against vertical transmission of BKD when injected into broodstock. However, these are not yet FDA-approved uses of the drug.

3. Sulfadimethoxine & Ormetoprim are available as Romet-30 and Romet TC (Aquatic Health Resources), which are two forms of the same potentiated antibiotic approved for use in salmonids for furunculosis and in catfish for enteric septicemia caused by *Edwardsiella ictaluri*. Both contain 25% sulfadimethoxine and 5% ormetoprim per pound and are added to medicated fish feeds available from various feed suppliers. The drug is administered at a dose of 50 mg per kg fish for 5 days for both salmonids and catfish with withdrawal times of 42 days for salmonids and 3 days for catfish. The drug has been effective in treating Oxytetracycline Dihydrate-resistant furunculosis as well as ERM but treatment for the latter disease is not an FDA-approved label use.

4. Chloramine-T (Halamid Aqua, Axcentive SARL; Western Chemical) is a new NADA approved drug for immersion application to control mortality in freshwater-reared salmonids due to bacterial gill disease caused by *Flavobacterium* sp at a dosage of 12 20 mg per L administered for 60 min daily in a static or flow-through bath on three consecutive or alternate days. Also approved to control mortality caused by external columnaris disease caused by *Flavobacterium columnare* in walleye at 10–20 mg per L and all freshwater-reared warmwater finfish at 20 mg per L administered for 60 min daily in a static or flow-through bath on three consecutive or alternate days.

5. Chorulon (Intervet Inc.) is a chorionic gonadotropin injectable used on male and female brood fish to enhance or promote spawning. Dosages are 50–510 IU per lb for males and 67–1,816 IU per lb for females. Allowed are up to three doses by intramuscular injection not to exceed 25,000 IU in fish intended for human consumption. There is a zero day withdrawal period and the drug must be prescribed by a licensed veterinarian.

6. Erythromycin Thiocyanate (Bimeda) can only be used by facilities that have been approved for use of INAD permit #6013 administered by the University of Idaho through the FDA or by extra-label veterinary prescription. The drug is an antibacterial against *Renibacterium salmoninarum* (Rs) used as a feed additive that has been available from various feed companies at doses of 100–200 mg per kg fish (0.3 to 3% of diet) originally administered for 14 days with 5 days of withdrawal and 7 more days of treatment. Additional regimens have been used including continuous 28-day feeding and intermittent feeding strategies of every other day for up to 60 days to increase drug absorption at colder water temperatures. The original target species on this permit included coho, Chinook, pink and sockeye salmon as well as eight other trout species. However, a recent misinterpretation by FDA has restricted drug use to Chinook salmon which is currently being reviewed for reinstating the other species. Because the vendor has shown fading interest in further drug development, alternative drugs are under investigation.

7. Erythromycin injectable (Erythro-200, Bimeda) Inactive INAD. Previously for approved users of INAD #6340, and injected (IP or dorsal sinus) into adult salmonid broodstock (species listed above) no later than 15 days prior to spawning in 1 or more doses each of 5–40 mg per kg body weight. Bactericidal levels of the drug accumulate in the egg yolk if injected prior to ovulation and appear effective in killing Rs cells in the egg. This regimen in

concert with prophylactic dietary application of the drug to early feeding fry hatching from eggs of injected parents has been successful in controlling vertical transmission of the Rs bacteria. Injected broodfish cannot be used for human consumption.

8. Tricaine Methanesulfonate (MS-222; Tricaine-S, Western Chemical; Finquel, Argent Laboratories) is a general anesthetic for amphibians, fish and other cold-blooded aquatic animals for temporary immobilization as an aid in handling, during manual spawning, weighing, measuring, marking, surgical operations, transport and photography. The drug is approved for use on fish in the families of Ictaluridae, Salmonidae, Esocidae and Percidae. The drug is dissolved in ambient water at a concentration of 15–330 ppm depending upon the degree of sedation desired, species and size of fish, water temperature, and softness—all of which determine drug efficacy. There is a withdrawal time for fish of 21 days. For use in aquatic amphibians and other aquatic poikilotherms the drug is used at 1:1000 to 1:20,000 depending upon the species and life stage of the animal and is limited to use in laboratories or hatcheries at water temperatures exceeding 10°C (50°F).

9. AQUI-S E and AQUI-S 20E (Aqua Tactics Fish Health) is a general anesthesia for a variety of fish used for temporary immobilization to aid in handling. Use of the drug requires an INAD permit (#11-741) with a dosage range of 10–100 mg eugenol per L with a recommended dosage of 20–30 mg eugenol per L to produce sedation reasonable for handling. Treatment duration is up to 15 min as a bath which will vary dependent on species, water temperature and level of sedation required. Use in a hatchery requires a 72 hr withdrawal holding period which is **not required for field use** involving fish not susceptible to legal harvest.

10. Formalin (Formalin F, Natchez Animal Supply Co.; Parasite-S, Western Chemical, Inc.; Formacide-B, B.L. Mitchell, Inc.) is an aqueous solution of 37% by weight formaldehyde gas used as a parasiticide on all finfish for external protozoa (*Ichthyophthirius* sp., *Chilodonella* sp., *Ichthyobodo* sp., *Schyphidia* sp., *Epistylis* sp., *Trichodina* sp.), monogenetic flukes (*Cleodiscus* sp., *Gyrodactylus* sp., *Dactylogyrus* sp.) and as a fungicide for Saprolegniaceae on all finfish eggs. As a parasiticide for all salmon and trout in tanks and raceways above 50°F, a drip of up to 170 µL per L for up to 1 hr; below 50°F, a drip for up to 250 µL per L for up to 1 hr; all other finfish a drip up to 250 µL per L for up to 1 hr; earthen ponds, 15–25 µL per L indefinitely. All finfish eggs are treated with a drip at 1,000–2,000 µL per L for 15 min except species of the order Acipenseriformes, which receive a drip for up to 1,500 µL per L for 15 min.

    Formalin is also used to control external protozoan parasites (species of the genera *Bodo, Epistylis*, and *Zoothamnium)* of penaeid shrimp. For shrimp in tanks and raceways 50–100 µL per L for up to 4 hr daily; shrimp in earthen ponds receive 25 µL per L as a single treatment.

    General restrictions for formalin use are (a) the chemical must not be subjected to temperatures below 40°F; (b) it cannot be applied to ponds when water is warmer than 80°F, there is a heavy phytoplankton bloom, or dissolved oxygen is less than 5 mg per L; (c) ponds may be retreated in 5 to 10 days if needed; (d) ponds containing striped bass cannot be treated; (e) it must be tested on a small number of animals or eggs from each lot to check for any unusual sensitivity to formalin before proceeding; and (f) there is 0-day withdrawal time.

Treatment of fish can be daily until parasite control is achieved; however, every-other-day treatments are often necessary to minimize gill hyperplasia from formalin exposure. Although there is no withdrawal time required for formalin, a 4- to 7-day withdrawal period prior to egg hatching and prior to seawater introduction of smolts may be necessary to assure successful transitions through these life stages.

Formalin should be in a closed container and stored in a safe area as described in the Fisheries Rehabilitation, Enhancement and Development manual *Safer Chemical Use In Alaskan Aquaculture* (ADF&G 1988). If stored at temperatures below 40°F formalin will develop a white precipitate of paraformaldehyde that is more toxic than the parent chemical. When this occurs, the aqueous portion is still usable but less potent. Because formalin is a strong oxidizer, it should not be used on fish when dissolved oxygen levels are 5 ppm or less.

11. Hydrogen Peroxide (35% PEROX-AID®, Western Chemical, Inc.) is approved for freshwater-reared finfish eggs to control mortality due to Saprolegniasis. The dosage for coldwater and coolwater eggs is 500–1,000 mg per L for 15 min in a continuous flow system once per day on consecutive or alternate days until hatching. Warmwater species receive 750–1,000 mg per L for 15 min in a continuous flow system once per day on consecutive or alternate days until hatching.

    The drug is also approved for use on freshwater-reared salmonids to control mortality due to bacterial gill disease associated with *Flavobacterium branchiophilum* at a dose of 100 mg per L for 30 min or 50–100 mg per L for 60 min once per day on alternate days for three treatments.

    A third approved use of the drug includes use on freshwater-reared coolwater finfish and channel catfish to control mortality due to external columnaris disease associated with *F. columnare*. The dosage for fingerlings and adults is 50–75 mg per L for 60 min once per day on alternate days for three treatments; the dosage for fry is 50 mg per L for 60 min once per day on alternate days for three treatments.

    Recommendations for drug use include performing an initial bioassay on a small number of fish before treating the entire group. The drug should not be used to treat northern pike or paddlefish (any life stage) or pallid sturgeon (fry). The drug should be used with caution on walleye. There is 0-day withdrawal time.

Selected chemicals of low regulatory priority (LRP): not approved by the FDA but currently allowed for use on food-fish without an INAD

1. Povidone Iodine *compounds* are used widely in fisheries as general disinfectants for utensils and as drugs when used for disinfection of eggs during or after water hardening. Products such as Wescodyne, Betadine, Ovadine and Argentyne are effectively used at 25–50 ppm for general disinfection and at 100 ppm for 10 min as external egg disinfectants or for 1 hr for both external and internal disinfection of eggs during water hardening. Argentyne and Ovadine are sold as buffered compounds but the other iodophors must be buffered to pH 7.0 with sodium bicarbonate. Alaska Title 16 regulations (5 AAC 41.080(b)) require iodophor disinfection of all fish eggs within 24 hours when transported between watersheds for at least 10 minutes with 100 ppm of active iodine ingredient at a pH of 6.0 or greater. The ADF&G fish disease policy requires that all eggs taken into the hatchery be surface disinfected as above regardless of watershed source. Exemptions include multimillion pink and chum egg

facilities where surface disinfection of so many eggs from the hatchery watershed may be impractical and unnecessary since egg-associated diseases have not been a problem in the broodstocks.

2. Sodium Bicarbonate is used (0.05%) to buffer certain unbuffered iodophor compounds to a pH of 6.0 to 7.0 when used at the working dilution of 100 ppm for egg disinfection. Sodium bicarbonate dissolved in ambient water at concentrations from 142–642 ppm is also used as a means of introducing carbon dioxide into the water to anesthetize fish after a 5 min exposure.
3. Acetic Acid can be used as a parasiticide at 1000–2000 ppm for 1–10 min as a bath.
4. Carbon Dioxide *gas* bubbled through the water column can be used as an anesthetic most commonly used for euthanasia of Pacific salmon broodstocks.
5. Sodium Chloride (NaCl) and seawater have proved useful in the following instances:
    a. NaCl is used as an osmoregulatory enhancer or antistressor for fish transport at 0.5% to 1% dissolved in freshwater for an indefinite period or at 3% for 10–30 min as a parasiticide.
    b. NaCl mixed 1:1 with calcium chloride (CaCl) has been used as a formalin replacement for treating egg fungus at the Robertson Creek Hatchery in Port Alberny, British Columbia. Hatchery staff used the equal mixture of NaCl and CaCl dissolved in freshwater at a final concentration of 20 ppt for a 1 hr static bath on coho and Chinook salmon eggs. Results were successful based on green egg to swim-up survivals when eggs were treated three times a week. However, the treatment costs were said to be expensive which may be prohibitive at most facilities.
    c. Seawater is more feasible in cost than NaCl for treating egg fungus at those facilities near saltwater access. Seawater has been used successfully for this purpose in at least two facilities in Alaska, i.e., Kitoi Bay and Armin Koernig Hatcheries. Raw seawater of 20–30 ppt is pumped to replace freshwater in the head boxes supplying incubators and then allowed to flow for a 1 hr exposure of pink and chum salmon eggs. Experienced temperature differences between fresh and seawater of 4°C to 6°C have not caused any adverse effects, and dissolved oxygen levels have been adequate. Some amount of fungus appears to buildup but not significantly enough to cause excessive egg mortality. Survivals from green egg to hatch in seawater-treated incubators have been equal to those in incubators treated for fungus by other means. Vibriosis or other fish diseases potentially originating from raw seawater have not occurred. However, UV depuration of the incoming raw seawater should be accomplished to control potential disease and nuisance organisms.
    d. Seawater at flows of 1–2 gpm mixed with 270 gpm of freshwater has also been used successfully to harden soft water from 0 to 300–500 units of conductivity at the Wally Noerenberg Hatchery. This has reduced coagulated yolk (white spot) and facilitates dissolving of eggshells at hatching for pink and chum salmon. Snettisham Hatchery recently switched from calcium chloride to UV-treated seawater for increasing water hardness. Recommended levels of water hardness for elimination of white spot are generally given in ppm ranging from 75–100 ppm. Raw seawater should be depurated with UV light prior to mixing in the hatchery water lines to prevent introduction of marine fish pathogens.

6. Calcium Chloride is also used to increase the hardness of water and has been used successfully at hatcheries such as Deer Mountain (Chinook, coho, steelhead), Crystal Lake (steelhead) and, prior to use of seawater, Snettisham (sockeye), to improve egg survival at hardness levels of 75–100 ppm. However, the annual cost is significant.

7. Sodium Sulfite has been used (not in Alaska) at a 15% solution for 5–8 min to improve hatchability of eggs. Further information on this use is vague.

Selected drugs used that are not FDA-approved

1. Quaternary Ammonium Compounds (Hyamine 1622, Roccal, Purina 4 Power) are used as footbath or utensil disinfectants at 600 ppm and have been used to treat bacterial gill disease of salmonid fishes at 1–4 ppm for a 1 hr flush for 2–3 consecutive days.

2. Diquat (1, 1'-ethylene-2, 2'-dipyridylium dibromide; Bipyridilium; Reglone) is an herbicide that has been used to treat bacterial gill disease of salmonids, generally at 2 ppm for a 1 hr flush for 2–3 consecutive days. Previously, Diquat was federally approved for use as an herbicide with food fish at 0.25–2.5 ppm having a withdrawal period of 14 days before treated water could be used for other purposes. Currently the compound is not FDA-approved but there is an INAD (#10-969) for its use to control external flavobacteriosis with a variety of dosages and exposure times available requiring 5 and 30 day withdrawal times unless fish cannot be legally harvested. The drug is EPA-approved for use on fungus with a 21-day withdrawal period.

3. Calcium Oxide (Quick lime) is considered LRP while calcium hydroxide (slaked lime) does not have that rating. Presently, these chemicals are not approved by the FDA. Previously, these compounds were GRAS by the FDA and were approved by the EPA as pond sterilants used at 1,338 lbs per acre (quick lime) and 1,784 lbs per acre (slaked lime).

4. Clove Oil and Eugenol are permitted by the FDA as food additives but may not be used in any form on fish that could be available for human consumption. Isoeugenol (AQUI-S) appeared to be a possible alternative but animal testing suggested the compound was carcinogenic and is now prohibited. AQUI-S-E and 20 E (50% eugenol) is a possible substitute under INAD (#11-741) exemption. A treatment dosage of 20–30 mg eugenol per L is recommended for all species to sedate for handling and requires a 72 hr withdrawal period if fish are available for harvesting and human consumption. If not, the withdrawal period is waived.

Surface disinfectants not used on fish

1. Didecyl-dimethyl Ammonium Chloride (Net-Dip) has been EPA approved as a general disinfectant and sanitizer used for fish holding equipment at 3.5 fl oz in 4 gal water for 10 min. The chemical is not to be used directly on fish or in water containing fish.

2. Calcium Hypochlorite (Olin HTH chlorinator) has been approved by the EPA as a disinfectant and sanitizer used for fish-holding equipment and utensils at 200 ppm of available chlorine on contact to disinfect and sanitize fish tanks, raceways and utensils. Substrate disinfection may require overnight exposure. HTH has also been used to destroy and disinfect fry that have undergone infection by IHNV. In this case the chlorine is administered to the raceway or tank of fish for a 6 hr exposure (ADF&G 1988). Other uses have been to disinfect (depurate) effluent and rarely influent water used in fish culture.

Effluent water is usually treated at 2 ppm residual chlorine for a minimum of 5 min contact time prior to dilution into surface water. Influent water has been treated successfully for viruses and bacteria at 1.2–1.6 ppm free chlorine for at least 1 min contact time prior to dechlorination by sodium thiosulfate used at about 0.56 g per 1 gal of chlorinated water. As a margin for error this is about 10 times more sodium thiosulfate needed to neutralize 1 gal of water containing 2 ppm free chlorine (ADF&G 1988). Compressed sulfur dioxide gas has also been injected into chlorinated water lines as a dechlorinator.

NOTE:

- Many of the compounds listed above are dangerous to human as well as fish health if used incorrectly. For additional information on safe chemical use in aquaculture refer to Wood (1979), OSHA guidelines, MSDS forms supplied with all chemical products and the Fisheries Rehabilitation, Enhancement and Development manual *Safer Chemical Use in Alaskan Aquaculture* (ADF&G 1988).
- Drug treatment calculations should be checked by at least two people as assurance against possible mathematical errors.
- External drug treatments, such as the use of formalin, should be done on a small group of fish first as another check for the accuracy of calculations and to reveal any unexpected adverse reactions of fish due to unknown variables.
- Application of any drug for the treatment of a suspected fish disease should not be done without positive identification of the problem and a recommendation from ADF&G fish pathology staff.

Nonchemical disinfection

Whenever possible, steam cleaning should be and has been substituted for chemical disinfection of raceways, fish tanks, floors and walls of buildings. Substrates and incubators have been disinfected by steam as well as by industrial washing equipment and detergent. Thorough rinsing must be observed when detergents are used.

DISINFECTION PROCEDURES FOR HATCHERIES

Egg disinfection

Introduction: Disinfection is necessary to control the spread of pathogens carried on the surface of eggs. Disinfection is done immediately after fertilization and during or after water hardening upon arrival and prior to exposure to running water at the receiving station. Eggs that have not been disinfected must not be placed into water at the receiving station unless the water can be held and sanitized before release. Otherwise, eggs should be returned to the point of origin or destroyed. Disposal should be by burial in dry ground or in wet ground with quicklime. Disinfection should also occur when eggs are taken at any site where incubation will occur (Wood 1979).

Products: ADF&G does not endorse any particular supplier or brand except in those instances where they are the only distributor or product approved for fisheries use.

- Betadine: (Purdue Products L.P.). Nondetergent, with 10% povidone iodine, aqueous polyvinyl pyrrolidone-iodine (1%). Not buffered. (Amend 1974; Vestal Laboratories, 1978)

- Wescodyne: (Steris Corp. Calgon Vestal Division). Detergent, with 1.6% active iodine in ethanol-iodine complexes. Not buffered. (Amend 1974; Vestal Laboratories, 1974)
- Argentyne: (Argent Chemicals). Nondetergent polyvinyl pyrrolidone iodophor similar to Betadine, but buffered.
- Ovadine (Syndel, Western Chemical). A nonstaining, noncorrosive buffered 10% polyvinylpyrrolidone-iodine complex (PVP Iodine) which provides 1% available iodine.

Methods: (Wood 1979; ADF&G 1983).

- Betadine, Argentyne or Ovadine: 1:100 dilution of jug strength for 10 min (100 ppm iodine).
- Wescodyne: 1:150 dilution of jug strength for 10 min (100 ppm iodine).
- Disinfect before exposing to running water at the receiving station, even when the egg take occurs at the receiving station.

Comments: To avoid the toxic acidifying effect from soft water, buffer Betadine and Wescodyne with 0.05% sodium bicarbonate.

Change iodophor solution between lots of fish or when it begins to lighten in color. A lot is defined—with respect to a pathogen or event in the influent hatchery water—as a group of fish of the same species and age that originated from the same discrete spawning population and share a common water supply within the hatchery. It may become necessary to compartmentalize a single lot as defined above into separate lots based on separate water manifolds to individual rearing containers having separate utensils.

Equipment sanitization

Introduction: The prevention of serious diseases caused by infectious agents at any hatchery is of utmost importance. Fish disease agents occur in hatcheries by the introduction of pathogens from egg, fish or equipment transfers and from populations of resident fish in the hatchery water supplies. Therefore, any interhatchery activities increase the concern for maintaining adequate disinfection and control of endemic diseases that may occur at those facilities.

Methods (Hnath 1983): All equipment used in one hatchery should not be allowed to enter any other hatchery until that equipment has been sanitized. Ideally, sanitation should occur before equipment leaves its resident station and again on its arrival at a second station. Equipment includes fish rearing containers, incubators, nets, fish pumps, utensils, raingear, waders, boots, egg sorters, fish transport vehicles, or anything else that may have had contact with fish, eggs, or culture waters. If fish transport motor vehicles are exchanged between facilities, they must be disinfected accordingly in a thorough manner to ensure efficacy.

1. *Rearing containers*: 200 ppm active chlorine in liquid bleach (sodium hypochlorite, 5.25% active ingredient) or calcium hypochlorite (HTH, registered, 65% active ingredient chlorine) for a minimum of 10 min. After disinfection, the solution should be dumped at a safe site where it will not directly drain into natural waters. Neutralization of chlorine is recommended by using 2 lb sodium thiosulfate per lb HTH or 1.5 g sodium thiosulfate per liter of 200 ppm chlorine. Chlorine is corrosive to metal and should be thoroughly rinsed with clean, uncontaminated water. Raingear should be worn to prevent or reduce chlorine

contact with clothing. Because organic substances will readily inactivate chlorine and limit its effectiveness, dirty equipment should be cleaned before it is disinfected with chlorine.

2. *Fish transport vehicle exterior*: The exterior of motor vehicles including chassis and undercarriage is decontaminated with high temperature (115–130°C) steam or with 20 ppm chlorine. Chlorine should be thoroughly rinsed with clean, uncontaminated water to minimize corrosion. It is not necessary to disinfect the exterior of aircraft or boats used for transporting fish or eggs.
3. *Fish transport vehicle interior*: Interior surfaces of motor vehicles, aircraft, or boats that have been contaminated during transport by contact with fish, eggs, or culture waters should be scrubbed with noncorrosive 600 ppm quaternary ammonia compounds, i.e., Hyamine or Roccal using 1.5 ml of 50% stock solution per L water; Iodophors at 100 ppm or Roccal at 800–1000 ppm for 30 min are the disinfectants of choice for transport tank interiors rather than chlorine solutions—which can adversely affect pumps and aerators.
4. *Other equipment*: Incubators, utensils, fish pumps, nets, egg sorters, waders, boots, raingear, etc., can be disinfected with 200 ppm chlorine for 10 min, 600 ppm quaternary ammonium compound for 30 min, or 100 ppm iodophor solution for 10 min. It may be necessary to scrub the disinfectant onto the surface. Disinfected equipment should be thoroughly rinsed with clean, uncontaminated water and dried before use.
5. *Personnel*: All individuals involved in transport operations should wear outer protective garments (rubber gloves, rain gear, boots, waders, etc.) when handling fish, eggs, or cultural water. Hands should always be disinfected before handling culture water at another station. When work is completed at the station, hands and protective garments should be properly disinfected. Natural cotton and wool fabrics that contact culture water at a station can be disinfected by soaking for 30 min in 600 ppm quaternary ammonia compound and then rinsed thoroughly before being worn.

Disinfectants are toxic to humans as well as fish. Care and common sense must be applied in their use to avoid upper respiratory irritations or contact dermatitis from continued overexposure. All containers of disinfectant must be capped or have lids on when not in use. The recommended levels for disinfection must not be exceeded. Disinfectants should be applied with brushes rather than aerosolized in a closed area. Goggles and respirators appropriate for the chemical used are necessary if aerosolization or splash will occur during chemical application. Live steam from a portable steam generator should be used for disinfection whenever possible to reduce chemical use.

Complete hatchery sanitization

Introduction: Plans for sanitizing a hatchery should be incorporated into the design of the facility such that, when and if necessary, disinfection can be accomplished easily and effectively.

Planning. Personnel designated to conduct the sanitization should formulate a detailed plan prior to the operation. This should include inspection of the facility, discussions with the manager, methods, materials, safety, training, and adequate follow up. Methods should include drying, elimination of water leaks or potential sources of contamination, volumetric measurements of the buildings, purchase of chemicals, initial cleaning, ventilation, and preventive maintenance.

Methods:

1. *Cleaning*: Most pathogens are removed from environmental surfaces by cleaning. Surfaces must be cleaned of dirt and organics for disinfectants to be effective.
2. *Drying*: Most fish pathogens (except infectious pancreatic necrosis virus) are destroyed by drying, thus, most anything that is clean and dry is generally free of viable agents. Some materials may be dry on the surface but not within. For example, wood may be dry on the surface, but wet internally. Concrete raceways can have cracks where water remains.
3. *Design*: A hatchery should be designed to allow maximal cleaning and drying of surfaces. The use of wood must be avoided and concrete floors should be sloped for adequate drainage and drying. Gravel floors cannot be adequately sanitized. Walls sealed with waterproof paint would also make later sanitation easier. Separate water manifolds supplying egg and rearing containers for different fish stocks and age groups of fish also help prevent pathogen spread via water.
4. *Wood*: Equipment and containers made of wood or other porous material used in the hatchery cannot be adequately disinfected and should be burned rather than attempting to reuse after sanitizing. Wooden incubators or rearing containers coated with fiberglass resin—although better than uncoated wood—should also be eliminated. Disinfection is still unreliable because minor delamination or cracking of the fiberglass is often difficult to detect.
5. *Concrete raceways*: Raceway sanitization is best accomplished by soaking in chlorine. First, look for cracks and leakage into and from other raceways and repair accordingly. Any significant amount of curing compounds, sealer or new concrete applied to a raceway surface for repair may require an undefined amount of time to leach out toxic compounds in running water before fish can safely inhabit the raceway. When in doubt, test a small number of fish in the raceway for at least 48 hours.
6. *Aluminum raceways*: Outside spraying with steam or chlorine (with proper respirator) rather than soaking will suffice since aluminum is nonporous. Gasoline or electrically powered high pressure sprayers have been very effective at some facilities for cleaning raceways (and other equipment) prior to disinfection.
7. *Fiberglass containers*: These should be considered as semiporous due to cracks that are often too small to be noticed. Spraying disinfectant may not be sufficient and soaking is preferred.
8. *Artificial substrate*: Saddles or biorings should be precleaned of organic debris and disinfected in chlorine for at least 30 min, rinsed in clean water and thoroughly dried before reuse the following season. After prolonged use, substrate will develop a surface scum that can be removed prior to disinfection by (1) agitation with sand in a cement mixer, (2) pressure spraying with water using commercial equipment, or (3) soaking in a citric acid solution for 24–48 hr.
9. *Disinfectants*:
    a. Steam: Steam should be used whenever possible to minimize use of toxic chemicals.
    b. Chlorine (with adequate respirator): 200 ppm chlorine can be used as a soak or as a spray for disinfection. Active available chlorine from HTH is about 65% (check label). A raceway should be filled halfway with water followed by addition of half the HTH accompanied by stirring (Hnath 1983). The raceway is then filled to within 5 cm of the top with water and the final half of the HTH is stirred in. Fill all raceways in the same

manner and include chlorination of all pipelines, especially drains. If possible, the entire raceway system should be disinfected at the same time. If the hatchery is too large to allow simultaneous disinfection it can be done in sections, being careful not to permit contaminated water to backflow into areas or pipelines already disinfected. The goal is to retain a level of 200 ppm chlorine in the raceways and lines for 1 hr and at least 100 ppm for several hours. Letting the raceways soak overnight is the safe way to do this. Sodium thiosulfate applied at 0.7 g per L provides the necessary quantity of sodium ions needed to neutralize the chlorine ions at 200 ppm strength after disinfection. Sufficient sodium thiosulfate should be on hand before chlorination begins so that an accident can be neutralized before an environmental disaster occurs. Allowing the chlorine solution to sit longer will permit enough chlorine molecules to escape into the atmosphere so that mixing or solubility variables will be more than compensated for. A recommended level of 1.5 g of thiosulfate per L errs on an excessive concentration to ensure complete neutralization of the chlorine. Measuring the residual chlorine (orthotolidine reagent or iodometric titration) after neutralization should be done to be certain that toxic levels are not released into the environment. Drinking water often contains 0.l ppm, which is sufficient to kill fish. Chlorine should not be detectable in effluent water.

c. Formalin fogging: Formalin fogging or fumigation is NOT recommended for human health reasons. Formalin fogging will produce a precipitate on every surface that dries, leaving a paraformaldehyde film. Paraformaldehyde sublimates slowly into the atmosphere as formaldehyde gas, leaving hazardous fumes in the hatchery for unpredictably long periods of time. Formalin fumigation using potassium permanganate can potentially produce a violent explosion and resultant formaldehyde gas is extremely dangerous in closed areas.

d. Iodophors: Disinfection with iodophor solutions containing 100 ppm available iodophor will suffice for walls, floors, and other nonporous surfaces.

e. Quaternary Ammonium Compounds (Roccal, Hyamine, etc.): Follow manufacturer's recommendations for use, but these compounds can be very toxic to fish and must be thoroughly rinsed from equipment before use.

10. *Respirators/Protective Clothes*: These should be worn whenever formalin, iodophor, chlorine, or other toxic chemicals are used, particularly in any manner that might cause aerosolization or splash. Respirators may be necessary during formalin treatments of eggs for fungus control. Knowledge of proper respirator use and assurance of proper function must be established before an individual performs tasks that require respirators. The correct respirator cartridges must be selected with regard to the toxic substances used.

11. *Environment*: Prior to sanitizing a raceway or any structure that will require large quantities of toxic chemicals, a failsafe plan must be designed that prevents environmental contamination. A second person should independently assess the plan and repeat the mathematical calculations.

FINFISH AND SHELLFISH DIAGNOSTIC PROCEDURES

Diagnostic procedures for the detection of finfish and shellfish diseases are described in a separate document, the *ADF&G Fish Pathology Section Laboratory Manual* (Meyers 2009).

HATCHERY INSPECTIONS

Annual or biannual hatchery inspections by a fish health professional evaluate facility design and practices as they relate to the control of fish and shellfish diseases. The function of the inspector is to offer advice to correct perceived fish health problems. A hatchery inspection includes an onsite visit and a written report submitted to the hatchery manager addressing the criteria listed below, much of which is provided by the hatchery manager through completion of several pre-inspection forms.

1. Fish stocks at facility (eggs or rearing fish): 1) number, 2) brood year, 3) source, 4) release dates, and 5) release locations.
2. Incubator types (fish species, loading densities, and percent survival-to-eyed stage).
3. Rearing containers (fish stock and species, size, and loading densities).
4. Water flow: 1) volume, 2) single pass, 3) re-use details (treatment, number of passes, etc.), 4) recirculation details (treatment, number of passes, etc.), 5) water source, 6) resident fish, 7) depuration (influent or effluent and method), 8) water temperature (at time of inspection), 9) source for water hardening of eggs, and 10) total dissolved gas.
5. Methods of fish movement from incubators to rearing to release.
6. Disinfection procedures (methods and dose) for: 1) eggs (before entering hatchery); 2) substrate (after each season); 3) utensils (between stocks); 4) equipment and incubators (between stocks or after each season); 5) footbaths in and out of facility; and 6) mortality disposal.
7. Current type of feed; 1) brand, 2) method of storage, and 3) turnover time (expiration dates, lot numbers).
8. Health problems observed in eggs or fish or both at facility; 1) stock lot, 2) age, and 3) signs.
9. Previous problems: 1) water quality (pH, temperature, sediment, dissolved oxygen concentration, total dissolved gases, hardness, etc.); 2) percent egg or fish mortality/stock or lot/day; 3) previous treatments for fungus control (chemical, dose, schedule), or other prophylactic or therapeutic treatments (reason, when, lot or stock, drug or chemical, method of application, dose, and results); and 4) feed; a) feed type, b) problem (odor, texture, palatability to fish, etc.), c) date, and d) lot number.

GOOD FISH CULTURAL PRACTICES AND ENVIRONMENT TO REDUCE DISEASES

Many diseases, both infectious and noninfectious, can be prevented by good fish cultural practices (ADF&G 1983) and a clean adequate water environment. Both of these requirements either eliminate pathogens or reduce stressors which predispose fish to diseases. This discussion assumes use of a water supply having adequate physical and chemical parameters for rearing salmonids. Some variables that can and should be optimized are listed:

1. *Adequate water flows.* Avoid *dead spots* or air pockets, especially in incubators. Any areas of no or low flow within incubators can result in localized egg or fry death and fungus buildup. Mortality and fungus continue to spread resulting in excessive ammonia which promotes more fungus and mortality that may destroy an entire incubator of fish and those below if in a stack.

2. *Proper egg and fish loading densities.* For various incubators and rearing containers, this is determined by volume and flow or other water quality parameters that might be unique to certain facilities.

3. *Proper feed pellet size.* Proper pellet size is used according to the fish life stage and percentage of body weight and volume fed according to water temperature. Feed with too many fines or too much feed too often can cause serious gill irritation, especially in Chinook salmon. Dry feeds are generally abrasive for starting Chinook fry. Overfeeding fish when water temperatures are very low is another common mistake made by hatchery staff. When water temperatures are 1°C to 2°C fish can be fed very low levels every other day without any adverse effects. Longer periods of starvation have been tested without problems occurring. Overfeeding at low water temperatures can cause gill irritation and excessive body or visceral fat that may result in organ dysfunction and sudden death under stress.

4. *Adequate conditions for feed storage.* Use feed before expiration of shelf life to prevent loss of vitamins and nutritional deficiencies, mold growth and rancidity.

5. *Adequate dissolved oxygen without gas supersaturation.* Oxygen concentrations for sustaining life should be maintained without exceeding 100% concentration of total dissolved gases that would result in gas supersaturation. Gas supersaturation causes gas bubble disease which often predisposes fish to many other diseases that would otherwise not occur or remain subclinical. Air entrained through a break or pinhole in hatchery plumbing, snow melt, hydroelectric turbines and extreme high barometric pressure can cause gas supersaturation at hatcheries. Routine periodic monitoring of total dissolved gases should be done with a saturometer at various points in the water flow at all facilities. Quite often supersaturation is transient, producing spikes that will go undetected unless frequent monitoring is done. Oxygen contacting systems can be used to displace dissolved nitrogen but overall total dissolved gases, including oxygen, should not exceed 100%.

6. *Increase the hardness* of *very soft incubation water* (as described previously). Some hardness prevents white spot disease (coagulated yolk) in eggs and later resultant dropout in the fry.

7. *Adequate fungus control.* On eggs use saltwater or formalin. Excessive formalin treatment can also cause white spot disease.

8. *Adequate disinfection of eggs.* Use iodophor compounds prior to incubation or placing eggs into the hatchery water supply. Exceptions would be certain multimillion egg pink and chum salmon facilities where disinfection would offer no benefit due to lack of any significant egg-associated pathogens in the disease histories of such facilities.

9. *Use of disinfectant footbaths.* Place footbaths between fish stocks and between incubation and rearing areas. Footbaths are especially necessary for preliminary isolation of a diseased group of fish.

10. *Use of separate utensils.* Use for each fish stock or most optimally for each separate lot of fish. Alternatively, utensils can be kept in disinfectant at various stations such that their common use will not spread diseases among the various lots or stocks of fish.

11. *Stringent use of the sockeye salmon culture policy*. The key elements are a virus-free water supply, adequate general disinfection including water-hardening eggs in iodophor for 1 hour and compartmentalization of eggs and fry to contain losses when IHN occurs. More details

are provided in the *Sockeye Salmon Culture Manual*, ADF&G Special Publication No. 6 (McDaniel et al. 1994).

12. *Use of disinfectable materials.* Use nonporous containers for incubating eggs and rearing juveniles. Anything made of wood is unacceptable.
13. *Adequate cleaning of raceways.* Frequent cleaning eliminates detritus, feces and saprophytic fungi.
14. *Limit fish access to the hatchery water supply*. Fish access should be limited as much as possible, especially for anadromous species. If obligate fish pathogens are present that routinely cause disease in the hatchery, depuration of the incoming water supply should be considered.
15. *Avoid significant pinheading*. A proper feeding regime discussed earlier is preventative as well as mechanical removal of fry from bulk incubators if fish will not outmigrate volitionally. Fish must have enough yolk (about 3–5%) to successfully start on feed which is especially true for certain stocks of chum salmon.
16. *Fish Health/Condition Assessment* (Periodic Examination of Moribund and Healthy Fish). The first line of defense against disease or poor fish performance in general is a regular examination of fish by species and by lot within a species. This involves more than just casual observation during feeding. Such observation is important for noting behavior and mortality levels, water flows and water quality, etc., but closer examination requires the sampling of fish, both healthy and moribund, for external as well as internal scrutiny. If this is done routinely when fish are apparently healthy, then the hatchery staff will be prepared to detect any deviation from normal when it develops. These routine examinations allow adjustment of feeding and other environmental parameters producing less stress and healthier fish which prevents some diseases from occurring. The procedures for this fish health assessment have been developed for the past 20 years by Goede (1997) and are simple to perform. Briefly, 20 live fish are collected from each lot within a stock; blood is examined for hematocrit (percent of packed red cell volume), buffy coat (white blood cells) and plasma protein content followed by length/weight measurements for body condition. Next, fish are examined externally for appearance of eyes, gills, pseudobranchs, thymus, fins and opercula. Internal examination follows for observations of mesenteric fat, spleen, hindgut, kidney, liver, bile and gonads. From these observations the general health and quality of the fish can be determined by comparison to a large data base of information. Also, dead fish should be examined as well since they are often the best source of clinical signs if a disease is present. A computer program is available for entering the data, computing results and reporting of the fish health/condition assessment.

    The value of establishing a normal condition profile for fish at each hatchery cannot be overemphasized for early detection of dietary, water quality or infectious problems and overall improvement of fish quality. Healthy fish have fewer diseases which can be discovered early by condition profiling so that corrective action may be taken before the problem is out of control. Fish health/condition assessment procedures should be practiced at all hatcheries statewide.

Vaccines

Occasionally prophylactic drugs are necessary to prevent clinical infectious disease when the risk is high. There are several vaccines commercially available for prevention of bacterial diseases in

salmonids such as vibriosis, enteric redmouth, and furunculosis. Most of these are applied by immersion but injection has often been more effective for furunculosis vaccines. Practical application of vaccines for viruses in the U.S. has not yet materialized. Although DNA vaccines for IHNV have shown very good protection experimentally as well as commercially in Canada, these still require injection for efficacy (LaPatra et al. 2001, Brudeseth et al. 2013).

The most commonly used vaccine in Alaska is the immersion type for vibriosis used to reduce fish losses once in seawater netpens. Generally, the risk of vibriosis becomes significant when seawater temperatures reach 8°C and beyond. If vaccination is planned, the following variables should be considered. Although these are based upon available laboratory and field results, users should always consult product information from the manufacturer for specific details regarding dosage, optimum fish size and immunization period at a suggested water temperature.

1. Ideally, immunization should occur about 30 days prior to seawater introduction such that adequate time has elapsed for immunity to develop at a water temperature of 10°C to 12°C.
2. The larger the fish ($\geq$ 4 g), the greater the immunological competence.
3. Variables such as small fish size, stress, smoltification, disease, dramatic fluctuations in water temperature, cold water temperatures, high suspended solids, improperly formulated diets and algal blooms can impair the development of adequate immunity.
4. Under optimum field conditions immunity may last from 9 mo to 1 yr, but generally the protective period is much less due to stress, etc. This is particularly true for Chinook salmon.
5. Despite vaccination, fish losses of up to 10% can occur from vibriosis even if ideal immunization conditions were apparent due to individual variation in immunocompetence.
6. Revaccination in seawater may be necessary, especially for second year Chinook salmon.

Recognition of disease at the hatchery

1. Keep containers of sick fish as isolated as possible, reducing potential exposure and spread of the disease to healthy lots should the cause be infectious.
2. List the clinical signs observed.
3. Note the environmental history, i.e., can these signs be related to water quality, handling, feeding, prior treatment for disease, etc., that hatchery staff can correct or account for?
4. Make an external examination of affected fish noting any gross lesions. Include wet mounts of gills, skin scrapes, and lesion material (if present) for examination with a compound microscope.
5. Make an internal examination of affected fish noting any gross lesions in the viscera, i.e., hemorrhage, pale coloration, discolored or white foci, ascites and foreign bodies. Include impression smears of lesion material, gut contents and blood for examination with a compound microscope.
6. Note any organisms observed during the external and internal examination of affected fish, i.e., protozoa, bacteria, helminth parasites, etc.

   Several manuals are available that describe and illustrate normal fish anatomy and the common fish health problems. Any of these would be helpful in directing preliminary fish health examinations. Recommended sources are *Diseases of Hatchery Fish* by James Warren (1991),

the *ADF&G Pathology Short-course Notebook* (ADF&G, 2013) and the *Fish Pathology Section Laboratory Manual* (Meyers, 2009) that provide detailed protocols for necropsy, sample collection and shipment as well as descriptive notes on common salmonid diseases in Alaska. Also available as a reference is the ADF&G fish pathology illustrated field guide to *Common Diseases of Wild and Cultured Fishes in Alaska* (Meyers et al. 2008).

7. Contact an ADF&G fish pathologist.

8. Be prepared to provide a complete recall of events (anamnesis) to the pathologist in charge. Fill out a case data report (contact the pathology staff). Information could include the following:

    A. Environmental history
        a. Water quality
            (1) Physical parameters (temperature, pH, dissolved oxygen concentration, salinity, runoff, etc.)
            (2) Source of water (well, river, reservoir)
            (3) Any recirculation or alternative water source used
        b. Nature of containment for fish (raceway, VR, pen, etc.) and hatchery layout regarding the number of different fish lots
        c. Other aquatic species present in the water source and their relative abundance.
        d. Any new change of hatchery procedure (new equipment, different disinfectant, change in diet, etc.)
        e. Any recent treatment for a fish health problem
        f. Any recent importations of fish or fish eggs onto the hatchery premises
        g. Type of diet used and storage practices
    B. Present clinical history
        a. Fish species, life-stage, brood year, source of stock, how many lots affected and loading densities
        b. Nature of disease
            (1) Acute or chronic
            (2) Clinical signs
                (a) Behavioral
                (b) Mortality rate
                (c) External lesions
            (3) Necropsy exam
                (a) How many fish examined
                (b) External observations, gross and microscopic
                (c) Internal observations, gross and microscopic

    Provision of as much information as possible by hatchery personnel will determine whether the fish health problem requires collection of samples for submission to one of the pathology laboratories. Complete preliminary information facilitates a more rapid response by pathology staff in the diagnosis of a fish health problem, especially since site visits to most hatcheries in Alaska are not likely on short notice due to the lack of roads and their remote locations.

9. Disease diagnosis by the fish pathologist is based on the results of the following actions.
    - Isolation of an infectious agent (fungal, bacterial or viral) if present in the samples examined, followed by molecular, biochemical or serological identification (definitive evidence). Protozoa and helminth parasites are generally identified according to their morphologies in wet mounts.
    - Clinical signs of disease (gross and microscopic tissue morphologies) and other anamnesis information (presumptive evidence).
    - Histopathology may be done if other observations and tests prove negative (usually presumptive evidence).
    - Transmission electron microscopy, optional and not usually routine (may also be definitive evidence).
10. Treatment, if appropriate, is determined by identification of the etiological agent or noninfectious cause and is recommended by the pathologist in charge.

PARTIAL LIST OF COMMON PATHOGENS FOR FINFISH AND SHELLFISH IN ALASKA

Tables 6–8 list common pathogens for finfish and shellfish disease in Alaska (for additional information see Piper et al. 1982; Elston 1990; Stoskopf 1993; Kennedy et al. 1996; Noga 1996; Elston 1999; Lewbart 2006; Bruno et al 2013).

Table 6.–Partial list of common pathogens for finfish in Alaska.

Pathogen		Explanation or species affected
Bacteria	1. *Renibacterium salmoninarum*	Bacterial Kidney Disease (BKD)
	2. *Aeromonas salmonicida* typical and atypical	Furunculosis
	3. *Aeromonas hydrophila/liquefaciens*	Motile Bacterial Septicemia
	4. *Pseudomonas fluorescens*	Motile Bacterial Septicemia
	5. *Pseudomonas* sp.	Motile Bacterial Septicemia
	6. *Vibrio (Listonella) anguillarum*	Vibriosis
	7. *Yersinia ruckeri* types 1 & 2	Enteric Redmouth
	8. *Serratia liquefaciens*	Bacterial Septicemia
	9. *Flavobacterium psychrophilum*	Coldwater Disease (sequela myeloencephalitis)
	10. Unidentified Flavobacteria	Superficial skin and gill infections
Fungi	1. *Saprolegnia* sp.	External egg and body fungus, internal systemic mycoses
	2. *Phoma* sp.	Internal infections of air bladder and other organs
Protozoa	1. *Trichodina* sp.	External gill and skin infections
	2. *Trichophrya* (*Capriniana)*	External gill infections (commensal)
	3. *Ichthyobodo(Costia) necatrix*	External gill and skin infections
	4. *Epistylis* sp.	External gill and skin infections
	5. *Myxobolus* sp.	Skin and internal infections in both fresh and saltwater fish species
	6. *Henneguya* sp.	Skin and internal infections in both fresh and saltwater fish species
	7. *Ceratomyxa shasta*	Internal infections of salmonids
	8. *Ichthyophonus* sp.	Internal granulomatous disease of marine species
Viruses	1. Infectious Hematopoietic Necrosis Virus (IHNV)	sockeye salmon and rarely, chum and Chinook salmon
	2. Viral Erythrocytic Necrosis Virus (VEN)	Pacific herring
	3. Viral Hemorrhagic Septicemia Virus (VHSV Type IVa)	Pacific herring, cod, hake, pollock
	4. Aquareovirus	Chinook salmon
	5. Paramyxovirus	Chinook salmon
	6. Erythrocytic Inclusion Body Syndrome (EIBS)	Chinook salmon
Noninfectious diseases or causes of mortality	1. Gas Bubble Disease	air entrainment, drop in barometric pressure, heating of very cold water
	2. Gill hyperplasia	feed or particulate abrasion, ammonia or formalin toxicity
	3. White Spot Disease	handling, soft water or aluminum toxicity
	4. Drop Out	too little yolk at swimup, sequela to white spot or not osmocompetent in seawater situations
	5. High egg or yolksac fry mortality	mechanical failure of incubator accompanied by ammonia toxicity and *Saprolegnia*; overloading, blank eggs or other developmental problem
	6. Excessive fat in body cavity or fatty liver or both	overfeeding during cold water temperatures
	7. Bloat	excessive feeding in seawater

Source: Meyers et al. 2008.

Table 7.–Partial list of common pathogens for bivalves in Alaska.

Pathogen		Species affected
Bacteria	1. *Nocardia crassostreae* (PON) in vesicular connective tissues, not common	Pacific oyster
	2. Rickettsial intracellular organisms in vesicular connective tissue cells, digestive tubule cells, gill epithelium and various other tissues	Pacific oyster weathervane scallop, blue mussel, clam species
Fungi	Systemic mycosis caused by unidentified fungus	basket cockle
Protozoa	1. *Ancistrocoma*-like ciliate in the digestive tubules and gut	Pacific oyster
	2. Unidentified small eosinophilic thigmotrich ciliate on the gills	Pacific oyster
	3. *Sphenophyra*-like ciliate on the gills	Pacific oyster
	4. Unidentified gregarines in gut, gills or otherwise histozoic	Pacific oyster, littleneck clam, cockle, blue mussel, scallops
	5. *Nematopsis* sp.	scallops, clams, mussel, cockle
	6. *Trichodina* sp. on gill and mantle epithelial surface	Pacific oyster
	7. *Hexamita* sp. within the tissues as secondary invaders	Pacific oysters
	8. Coccidia-like organisms in connective tissue and kidney	native littleneck clam, basket cockle.
	9. Microsporidia in ova, nervous tissue, connective tissue and muscle	clams, cockle
Metazoa	1. Unidentified copepods on the gills, in the digestive tubules, intestine and connective tissues (suggest *Pseudomyicola*)	Pacific oyster, littleneck clam, blue mussel, rock scallop, cockle
	2. Unidentified trematode metacercariae and sporocysts in connective tissues	blue mussel, razor clam, cockle, littleneck clam, weathervane scallop,
	3. Turbellaria—gill and gut	Pacific oyster, weathervane scallop, littleneck clam, cockle, blue mussel
Noninfectious anomalies	1. Pearls	Pacific oyster, blue mussel, weathervane scallop
	2. Hermaphroditism	potentially all bivalves; some are normally hermaphroditic
	3. Summer Mortality—stress related due to prolonged near-mature condition of gonads in both sexes	Pacific oyster (primarily females)
	4. neoplasia–	
	a) germinoma	Pacific oyster
	b) mesenchymal tumor	blue mussel,
	c) secretory cell adenoma	geoduck clam
Viruses	1. Viral gametogenic hypertrophy (Ovacystis)—papilloma/polyoma-like viruses in germinal cells of gonads	Pacific oyster
	2. Intranuclear Cowdry-type A inclusions of digestive tubule cells or mantle epithelium caused by a herpes-like virus	native littleneck clam, rock scallop, probably Pacific oyster
	3. Aquareovirus—probable bioaccumulated fish virus	geoduck clam
	4. Aquabirna virus—probable bioaccumulated fish virus	littleneck clam
	5. Disseminated neoplasia—leukemia caused by suspected retrovirus	blue mussel, native littleneck clam

Source: Meyers and Burton 2009.

Table 8.–Partial list of common pathogens for crabs in Alaska.

Pathogen		Species affected
Bacteria	1. Bacteremia, possibly from injury or stress	red, blue, golden king crabs; Dungeness crab; *bairdi* Tanner crab
	2. Rickettsial intracellular organisms in digestive gland epithelium	blue and golden king crabs
	3. Shell Disease: several Gram-negative bacterial species causing shell erosion	potentially all crab species
Fungi	Black Mat fungus *Trichomaris invadens*	Tanner crabs
Viruses	1. Herpes-like virus of bladder and antennal gland	red, blue and golden king crabs
	2. Aquabirna-like virus of antennal gland	blue king crab
Protozoa	1. Bitter Crab Dinoflagellate Syndrome—systemic	*bairdi* Tanner crab, *opilio* snow crab
	2. *Mesanophrys* ciliate—systemic	blue and golden king crabs, Tanner crab, Dungeness crab
	3. Haplosporidian-like organism systemic	spot and pink shrimps
	4. Microsporidia including *Thelohania* sp., various tissues	red, blue, golden king crabs, coonstripe shrimp

Source: Meyers and Burton 2009.

INVESTIGATION OF FISH KILLS

Objective

A pathology examination or necropsy of fish/shellfish may establish whether an infectious or parasitic cause of death is present and, within narrow limits, may be able to estimate approximate time of animal death based on gross and microscopic tissue changes. Depending on specific tissue changes present, a necropsy occasionally can provide clues suggesting that a fish kill was caused by noninfectious environmental trauma or intoxication. Accurate pathology interpretation is contingent upon receiving animal tissues that are in good condition and fresh. All animal tissues decompose soon after death, obscuring any abnormal tissue changes that might have been present in the living animal. Therefore, decomposed tissues are unacceptable for necropsy. Necessary information for investigation of fish kills includes the following:

Habitat assessment

1. Date and time of day observations made
2. Site location with a description of area affected including identifying landmarks and recent excavation, construction, or other activity present
3. Name, address, telephone number of person who first noted the fish kill
4. Names of other witnesses
5. Time when fish kill first reported
6. Estimated time when fish kill began
7. Water quality characteristics
    - Dissolved oxygen concentration
    - pH
    - Water temperature
    - Conductivity

- Color of water
- Odor of water
- Presence of algal blooms
- Salinity if seawater or estuary

8. Characteristics of the fish

    - Condition of fish observed (live, moribund, dead, decaying)
    - Size and species distribution of affected fish
    - Condition of the dead or moribund fish (gills flared, gaping mouths, fins extended, external lesions present on gills and skin, external parasites, excessively dark or abnormal coloration, spinal curvatures, excessive mucus, chemical odor, normal but dead, etc.)
    - Behavior of live or moribund fish (listless, prostrate, corkscrew swimming, convulsive, attempting to escape from water, flashing, gasping at surface, normal, etc.)

9. Characteristics of invertebrates

    - Condition of invertebrates observed (live, moribund, dead)
    - Species
    - Coloration and visible abnormalities
    - Behavioral abnormalities

10. Characteristics of plants (dead, discolored, normal, etc.) and sediments (discolored, bad odor, etc.)

11. Presence of obvious chemical or other foreign materials including description and sample of foreign condition

Collection of fish or invertebrate samples and sample materials

1. Optimum samples to collect are moribund (sick) fish/shellfish that must be kept refrigerated (do not freeze). If moribund fish are not available freshly dead will suffice. Package and label separately if both live and dead animals are collected (10 per group is usually sufficient). Decomposed animals are not useful for pathological examinations.

2. Live and fresh dead animals should be placed into Whirl-Pak or Ziploc bags. Samples must be kept cold in transit.

3. Place bagged animals into a cooler on gel ice with newspaper or other material in between for insulation to keep from freezing.

4. Live (moribund) animals that are 15 cm total length or less can be placed in jars with 10% buffered formalin for immediate fixation (5 animals). Abdomens should be dissected open and internal organs pulled out slightly. Allow 10 times more volume of fixative than tissue for proper fixation.

5. Larger fish require onsite excision of major tissues and internal organs. Approximately 1 cm square pieces of tissue are placed into 10% buffered formalin at a ratio of approximately 1 part tissue to 10 parts fixative.

6. A black waterproof magic marker is used to label plastic bags and tape on the outside of fixative jars. A label must also be included inside each fixative jar using a lead pencil to mark

a square of paper. All labels should include collection date, location of collection, and contents.

7. A pathology sample submission form must be included with each group of samples submitted. Place all paperwork and forms into a Ziploc plastic bag to keep dry and legible. Place in cooler with the samples.
8. Always call the fish pathology section staff at the nearest laboratory before sending samples. Send refrigerated or fixed samples or both to Anchorage Fish Pathology Laboratory, 333 Raspberry Road, Anchorage, AK 99518, 267-2244 or Juneau Fish Pathology Laboratory, 3333 Old Glacier Highway, Juneau AK 99801, 465-3577

Common mistakes to avoid when submitting pathology samples

1. Do not freeze the samples. Unintentional freezing can occur on gel ice if samples are not insulated with newspaper or other material. Freezing destroys tissue structure making most pathology interpretations impossible.
2. If samples are marine shellfish, use gel ice since regular ice (freshwater) will melt and must not come in contact with animal tissues. Freshwater contact with hypertonic tissues of marine nonregulators will cause water absorption, swelling and destruction of cellular integrity.
3. Do not put too much tissue into fixative jars. Allow for 10 times more fixative than tissue.
4. Abdomens of fish must be dissected open before placing into fixative to adequately preserve internal organs and tissues.
5. Decomposed samples are not acceptable. If tissues are discolored, soft and pull apart easily or have a putrid odor, they are decomposed and of no value for pathology examination.
6. Habitat assessment information and a pathology submission form must be included when submitting samples. This information is often the most important for solving the cause of a fish kill and can only be provided by the person collecting the samples.
7. Excessive numbers of animals should not be placed in a single sample bag. This can result in crushed tissues and incomplete chilling. Use common sense—5 fish per bag if small or single fish per bag if larger. Keep bags equally distributed in the cooler for shipment.
8. Live and dead animals should not be mixed. Keep them in separate bags and make sure everything is labeled properly.
9. Always call an ADF&G fish pathology staff member before submitting samples.

REFERENCES CITED

Amend, D. F. 1974. Comparative toxicity of two iodophors to rainbow trout eggs. Transactions of the American Fisheries Society 103(1):73–78.

ADF&G. 1983. Fish culture manual. Alaska Department of Fish and Game, Division of Fisheries Rehabilitation, Enhancement, and Development, Juneau, AK.

ADF&G. 1988. Safer chemical use in Alaskan aquaculture. Alaska Department of Fish and Game, Division of Fisheries Rehabilitation, Enhancement, and Development, Juneau, Alaska.

ADF&G Staff. 2013. Fish Health Workshop Notebook, Alaska Department of Fish and Game, Fish Pathology Section, Anchorage, Alaska, March 5-7.

Brudeseth, B. E, R. Wiulsrød, B. N. Fredriksen, K. Lindmo, K-E Løkling, M. Bordevik, N. Steine, A. Klevan, K. Gravningen. 2013. Status and future perspectives of vaccines for industrialized fin-fish farming. Fish & Shellfish Immunology 35:1759-1768.

Bruno D. W., P. A. Noguera, T. T. Poppe. 2013. A colour atlas of salmonid diseases. 2nd edition, Springer, New York.

DiMasi, J. A., R. W. Hansen, H. G. Grabowski. 2003. The price of innovation: new estimates of drug development costs. Journal of Health Economics 22:151–185.

Elston, R. A. 1990. Mollusc diseases: guide for the shellfish farmer. Washington Sea Grant Program, Seattle, Washington.

Elston, R. A. 1999. Health management, development and histology of seed oysters. World Aquaculture Society. Baton Rouge, Louisiana.

Emmenegger, E. J., T. R. Meyers, T. Burton, G. Kurath. 2000. Genetic diversity and epidemiology of infectious hematopoietic necrosis virus in Alaska. Diseases of Aquatic Organisms 40:163–176.

Goede, R. 1997. Goede health condition profile. Pages 21–44 [*In*] Fish Health Workshop Notebook, Alaska Department of Fish and Game, Fish Pathology Section, Anchorage, Alaska.

Hnath, J. G. 1983. Hatchery disinfection and disposal of infected stocks. Pages 121–134 [*In*] F. P., Meyer, J. W. Warren, T. G. Carey, editors. A guide to integrated fish health management in the Great Lakes basin, Great Lakes Fishery Commission, Ann Arbor Michigan. Special Publication No. 83-2.

Kennedy, V. S., R. I. E. Newell and A. F. Eble. 1996. The eastern oyster, *Crassostrea virginica*. Maryland Sea Grant College, College Park, Maryland.

Kurath G, K. A. Garver, R. M. Troyer, E. J. Emmenegger, K. Einer-Jensen, E. D. Anderson. 2003. Phylogeography of infectious haematopoietic necrosis virus in North America. Journal of General Virology 84:803−814.

LaPatra, S. E., S. Corbeil, G. R. Jones, W. D. Shewmaker, N. Lorenzen, E. D. Anderson, and G. Kurath. 2001. Protection of rainbow trout against hematopoietic necrosis virus four days after specific or semi-specific DNA vaccination. Vaccine 19:4011–4019.

Lewbart, G. A. 2006. Invertebrate medicine. Blackwell Publishing. Ames, Iowa.

McDaniel, T. R., K. M. Pratt, T. R. Meyers, T. D. Ellison, J. E. Follett, and J. A. Burke. 1994. Alaska Sockeye Culture Manual. Special Fish Report No. 6, Alaska Department of Fish and Game, Juneau, Alaska.

Meyers, T. R., T. Burton, C. Bentz, N. Starkey. 2008. Common diseases of wild and cultured fishes in Alaska. Alaska Department of Fish and Game, Division of Commercial Fisheries, Anchorage, Alaska.

Meyers, T. R., T. Burton. 2009. Diseases of wild and cultured shellfish in Alaska. Alaska Department of Fish and Game, Division of Commercial Fisheries, Anchorage, Alaska.

Meyers, T. R., editor. 2009. Fish pathology section laboratory manual. Special Publication No. 12, 3rd edition, Alaska Department of Fish and Game, Division of Commercial Fisheries, Juneau, Alaska.

REFERENCES CITED (Continued)

Noga, E. J. 1996. Fish disease; diagnosis and treatment. Mosby-Year Book, Inc., St. Louis, Missouri.

Ossiander, F. J. and G. Wedemeyer. 1973. Computer program for sample sizes required to determine disease incidence in fish populations. Journal of the Fisheries Research Board of Canada 30:1383–1384.

Piper, R. G., I. B. McElwain, L.E. Orme, J. P. McCraren, L. G. Fowler, and J. R. Leonard. 1982. Fish Hatchery Management. U.S. Department of Interior, Fish and Wildlife Service, Washington, D.C.

RaLonde, R. 1993. Shellfish aquaculture in Alaska and the potential of interaction with wild species. Proceeds from the 22nd US-Japan Aquaculture Panel Symposium, Homer, Alaska, August 21–22, 1993. Alaska Sea Grant Report AK-SG-95-03:27–39, Fairbanks, Alaska.

Simon, R. C. and W. B. Schill. 1984. Tables of sample size requirements for detection of fish infected by pathogens: three confidence levels for different infection prevalence and various population sizes. Journal of Fish Diseases 7:515–520.

Stefan, G. E. 1992. FDA regulation of animal drugs used in aquaculture. Aquaculture Magazine 18:62–67.

Stoskopf, M. K. 1993. Fish medicine. W. B. Saunders Company, Philadelphia, Pennsylvania.

True, K., editor. 2000. National Wild Fish Health Survey Laboratory Procedures Manual. 1st edition, U.S. Fish and Wildlife Service, Division of Hatcheries, Washington, D.C.

Vestal Laboratories. 1978. Notes on V.I.S. and other iodophors. Technical bulletin 85–201. St. Louis, Missouri.

Warren. J. W. 1991. Diseases of hatchery fish, 6th edition. U.S. Fish and Wildlife Service, Pacific Region.

Wood, J. W. 1979. Diseases of Pacific salmon, their prevention and treatment, 3rd edition. State of Washington Department of Fisheries, Hatchery Division.

APPENDIX

Appendix A.–Schedule I for fluorescent antibody test (FAT).

Rationale. Detection of disease-causing agents in fish populations becomes more difficult with covert existence in a carrier state. Subclinical infection produces no obvious external or internal signs of disease. Thus, destructive sampling of larger numbers of fish is required to reduce the risk of not detecting a disease organism with acceptable statistical confidence. The efforts and cost required to process such samples are considerable, and proportional to the number of samples. Consequently, it is imperative that sample numbers be as small as possible, but still provide statistically reliable prevalence data. The model that best fits most situations encountered in sampling fish for disease detection is the hypergeometric distribution (Ossiander and Wedemeyer 1973, Simon and Schill 1984). This model was used to compute Schedule I for all finite sample sizes. The binomial approximation to the hypergeometric distribution was used for the infinite population case (populations greater than 25,000).

The Schedule I used in this document for Rs (BKD), *A. salmonicida* and ERM agent screening consists of the last subtable where population size is infinite. Note that there is little change in the schedule as population sizes increase from 1000 to infinity. Sixty fish is the sample size providing a 95% confidence that at least a single diseased fish will be detected in the sample if disease is present within 5% of the population. Prerelease evaluations for BKD, ERM and *A. salmonicida* agents are performed with juvenile fish using the FAT. Results are recorded on a scale of 1+ to 5+ according to the intensity of fluorescence and relative numbers of organism in 30 microscope fields at 1000× magnification. The most conservative approach would be to reject a fish population if one fish tests positive in a sample of 60. However, a more practical compromise is necessary between the ideal situation of no disease and a more realistic one where some disease in the carrier state is frequently present and must be tolerated to some degree. That degree of tolerance (acceptable percent of positive FAT categories within the population) is arbitrarily determined in Schedule I (infinite population table), whereby, at a 5% risk of no detection in a 60-fish sample, the population is rejected (i.e., limitations may be placed upon the disposition of those fish as determined on a case-by-case basis) if 7 or more fish are 1+ by FAT (population prevalence of 20%); 2 or more fish are 2+ (population prevalence of 10%); or 1 or more fish are 3+ (population prevalence 5%), i.e., no 3+,4+, or 5+ fish are allowed due to the large numbers of disease organisms carried and potentially released into the environment.

Schedule I. Rejection numbers for different population and sample sizes when the risk is 5% (0.05).

Population Size = 1,000

	% Disease	Sample Size						
FAT	Prevalence	30	60	100	120	200	300	500
1+	20	3	7	14	17	32	51	90
2+	10	1	3	6	7	14	23	42
3+	05		1	2	3	6	10	19
4+	01						1	2

Population Size = 2,000

	% Disease	Sample Size						
FAT	Prevalence	30	60	100	120	200	300	500
1+	20	3	7	14	17	31	50	87
2+	10	1	2	5	7	14	22	41
3+	05		1	2	2	5	9	18
4+	01						1	2

Population Size = 5,000

	% Disease	Sample Size						
FAT	Prevalence	30	60	100	120	200	300	500
1+	20	3	7	14	17	31	49	86
2+	10	1	2	5	7	13	22	40
3+	05		1	2	2	5	9	18
4+	01						1	2

Population Size = 10,000

	% Disease	Sample Size						
FAT	Prevalence	30	60	100	120	200	300	500
1+	20	3	7	14	17	31	49	86
2+	10	1	2	5	7	13	22	39
3+	05		1	2	2	5	9	17
4+	01						1	2

Population Size = 25,000

	% Disease	Sample Size						
FAT	Prevalence	30	60	100	120	200	300	500
1+	20	3	7	14	17	31	49	86
2+	10	1	2	5	7	13	22	39
3+	05		1	2	2	5	9	17
4+	01						1	2

Population Size = infinite

	% Disease	Sample Size						
FAT	Prevalence	30	60	100	120	200	300	500
1+	20	3	7	14	17	31	49	85
2+	10	1	2	5	7	13	22	39
3+	05		1	2	2	5	9	17
4+	01						1	2

Appendix B.–Publications by Fish Pathology Section staff.

Arsan, E. L., S. D. Atkinson, S. L. Hallett, T. Meyers, and J. L. Bartholomew. 2007. Expanded geographical distribution of *Myxobolus cerebralis*: first detections from Alaska. Journal of Fish Diseases 30:483–491.

Bentz, C. R., C.I. Hogge, T. R. Meyers, J. A. Ferguson. 2012. *Myxobolus neurotropus* infecting rainbow trout *Oncorhynchus mykiss* in Alaska, a new geographic record. Journal of Aquatic Animal Health 24:225–231.

Boustead, N. C., T. R. Meyers, and S. Short. 1993. Absence of infectious haematopoietic necrosis virus (IHNV) in New Zealand sockeye salmon, *Oncorhynchus nerka*. New Zealand Journal of Marine and Freshwater Research 27:55–60.

Boustead, N., R. Pascho, D. Elliott, and T. Meyers. 1999. Data on New Zealand salmon samples examined for *Renibacterium salmoninarum* by United States laboratories. National Institute of Water and Atmospheric Research Ltd., Christchurch, NZ. Client report No. CHC99/8, Project No. SCF9053.

Burke, J., D. Mulcahy. 1983. Retention of infectious haematopoietic necrosis virus infectivity in fish tissue homogenates and fluids stored at three temperatures. Journal of Fish Diseases 6:543–547.

Burke, J., R. Grischkowsky. 1984. An epizootic caused by infectious hematopoietic necrosis virus in an enhanced population of sockeye salmon, *Oncorhynchus nerka* (Walbaum), smolts at Hidden Creek, Alaska. Journal of Fish Diseases 7:421–429.

Burton, T. O., T. R. Meyers, N. Starkey, J. E. Follett. 2004. Experimental transmission of the fungus *Phoma herbarum* to Chinook salmon. Journal of Aquatic Animal Health 16:251–257.

Carls, M. G., G. D. Marty, T. R. Meyers, D. E. Hinton, R. E. Thomas, and S. D. Rice. 1998. Expression of viral hemorrhagic septicemia virus in prespawning Pacific herring (*Clupea pallasi*) exposed to weathered crude oil. Canadian Journal of Fisheries and Aquatic Sciences 55:2300–2309.

Eaton, W. D., M. L. Kent, T. R. Meyers. 1991. Coccidia, X-cell pseudotumors and *Ichthyophonus* sp. infection in walleye pollock (*Theragra chalcogramma*) from Auke Bay, Alaska. Journal of Wildlife Diseases 27:140–143.

Eaton, W. D., D. C. Love, C. Botelho, T. R. Meyers, K. Imamura, and T. Koeneman. 1991. Preliminary results on the seasonality and life cycle of the parasitic dinoflagellate causing bitter crab disease in Alaskan Tanner crabs (*Chionoecetes bairdi*). Journal of Invertebrate Pathology 57:426–434.

Elston, R., C. Dungan, T. Meyers, and K. Reece. 2004. *Perkinsus* Sp. infection risk for Manila clams, *Venerupis philippinarum* (A. Adams and Reeve, 1850) on the Pacific coast of North and Central America. Journal of Shellfish Research 23:101–105

Elston, R., and T. R. Meyers. 2009. Effect of viral hemorrhagic septicemia virus on Pacific herring in Prince William Sound, Alaska, from 1989 to 2005. Diseases of Aquatic Organisms 83:223–246.

Emmenegger, E. J., T. R. Meyers, T. Burton, and G. Kurath. 2000. Genetic diversity and epidemiology of infectious hematopoietic necrosis virus in Alaska. Diseases of Aquatic Organisms 40:163–176.

Follett, J. E., J. B. Thomas, A. K. Hauck. 1987. Infectious haematopoietic necrosis virus in moribund and dead juvenile chum, *Oncorhynchus keta* (Walbaum), and Chinook, *O. tshawytscha* (Walbaum) salmon and spawning adult chum salmon at an Alaskan hatchery. Journal of Fish Diseases 10:309–313.

Follett, J. E., and M. K. Schmitt. 1990. Characterization of a cell line derived from inconnu. Journal of Aquatic Animal Health 2:61–67.

Follett, J. E., J. L. Geesin, and T. O. Burton. 1994. Detection of *Ceratomyxa shasta* in Alaskan chum salmon, *Oncorhynchus keta*. Alaska Fishery Research Bulletin 1:97–98.

Follett. J. E., and T. O. Burton. 1995. Epizootics of infectious hematopoietic necrosis virus in an enhanced population of sockeye salmon *Oncorhynchus nerka* smolts at Chenik Lake, Alaska. Alaska Fishery Research Bulletin 2:137–142.

Follett, J. E., T. R. Meyers, T. O. Burton, and J. L. Geesin. 1997. Comparative susceptibilities of several salmonid species in Alaska to infectious hematopoietic necrosis virus (IHNV) and North American viral hemorrhagic septicemia virus (VHSV). Journal of Aquatic Animal Health 9:34–40.

Goodwin, A. E., J. E. Peterson, T. R. Meyers, and D. J. Money. 2004. Transmission of exotic fish viruses: the relative risks of wild and cultured bait. Fisheries 29:19–23.

Grischkowsky, R. S., and D. F. Amend. 1976. Infectious hematopoietic necrosis virus: prevalence in certain Alaskan sockeye salmon, *Oncorhynchus nerka*. Journal of the Fisheries Research Board of Canada 33:186–188.

Hawkes, C. R., T. R. Meyers, T. C. Shirley, and T. M. Koeneman. 1986. Prevalence of the parasitic barnacle *Briarosaccus callosus* on king crabs of Southeastern Alaska. Transactions of the American Fisheries Society 115:252–257.

Hawkes, C. R., T. R. Meyers, and T. C. Shirley. 1987. Growth of Alaskan blue king crabs, *Paralithodes platypus* (Brandt), parasitized by the Rhizocephalan, *Briarosaccus callosus* Boschma. Crustaceana. 52:78–84.

Hershberger, P. K., R. M. Kocan, N. E. Elder, T. R. Meyers, and J. R. Winton. 1999. Epizootiology of viral hemorrhagic septicemia virus in Pacific herring from the spawn-on-kelp fishery in Alaska, USA. Diseases of Aquatic Organisms 37:23–31.

Hershberger, P. K., N. E. Elder, C. A. Grady, J. L. Gregg, C. A. Pacheco, C. Greene, C. Rice, and T. R. Meyers. 2009. Prevalence of viral erythrocytic necrosis in Pacific herring and epizootics in Skagit Bay, Puget Sound, WA. Journal of Aquatic Animal Health 21:1–7.

Kocan, R., M. Bradley, N. Elder, T. Meyers, B. Batt, and J. Winton. 1997. North American strain of viral hemorrhagic septicemia virus is highly pathogenic for laboratory-reared Pacific herring. Journal of Aquatic Animal Health 9:279–290.

Lovy, J., N. L. Lewis, P. K. Hershberger, W. Bennett, T. R. Meyers, K. A. Garver. 2012. Viral tropism and pathology associated with viral hemorrhagic septicemia in larval and juvenile Pacific herring. Veterinary Microbiology 161:66–76.

Marty, G. D., E. F. Freiberg, T. R. Meyers, J. Wilcock, T. Farver, and D. Hinton. 1998. Viral hemorrhagic septicemia virus, *Ichthyophonus hoferi*, and other causes of morbidity in Pacific herring *Clupea pallasi* spawning in Prince William Sound, Alaska, USA. Diseases of Aquatic Organisms 32:15–40.

Marty, G.D., T. J. Quinn II, G. Carpenter, T. R. Meyers, and N. H. Willits. 2003. Role of disease in abundance of a Pacific herring population. Canadian Journal of Fisheries and Aquatic Sciences 60:1258–1265.

McDaniel, T. R., K. M. Pratt, T. R. Meyers, T. D. Ellison, J. E. Follett, and J. A. Burke. 1994. Alaska sockeye salmon culture manual. Special Publication No. 6, Alaska Department of Fish and Game, Juneau, Alaska.

Meyers, T. R., A. K. Hauck, W. D. Blankenbeckler, and T. Minicucci. 1986. First report of viral erythrocytic necrosis in Alaska, USA, associated with epizootic mortality in Pacific herring, *Clupea harengus pallasi* (Valenciennes). Journal of Fish Diseases 9:479–491.

Meyers, T. R., T. M. Koeneman, C. Botelho, and S. Short. 1987. Bitter Crab Disease: a fatal dinoflagellate infection and marketing problem for Alaskan Tanner crabs *Chionoecetes bairdi*. Diseases of Aquatic Organisms 3:195–216.

Meyers, T. R., P. Krasnowski, D. Amend, B. Bachen, J. Cochran, K. Hauck, K. Rawson, and R. Saft. 1988. Regulation changes, policies and guidelines for Alaska fish and shellfish health and disease control. Special Fisheries Rehabilitation, Enhancement, and Development Division Report, Alaska Department of Fish and Game, Juneau, Alaska.

Meyers, T. R. 1989. Apparent chronic bacterial myeloencephalitis in hatchery-reared juvenile coho salmon *Oncorhynchus kisutch* in Alaska. Diseases of Aquatic Organisms 6:217–219.

Meyers, T. R. 1990. Diseases of Crustacea - diseases due to protistans and metazoans. Pages 350–389 [*In*] O. Kinne, editor. Diseases of Marine Animals Vol. III, Biologische Anstalt Helgoland, Hamburg, Germany.

Meyers, T. R., C. Botelho, T. M. Koeneman, S. Short, and K. Imamura 1990. Distribution of bitter crab dinoflagellate syndrome in southeast Alaskan Tanner crabs *Chionoecetes bairdi*. Diseases of Aquatic Organisms 9:37–43.

Meyers, T. R., S. Short, W. Eaton. 1990. Diseases of five commercial crab species in Southeast Alaska. Pages 407–420 [*In*] C. Cheng, F. Perkins, editors. Pathology in Marine Science, Academic Press, New York.

Meyers, T. R., S. Short, and W. Eaton. 1990. Summer mortalities and incidental parasitisms of cultured Pacific oysters in Alaska. Journal of Aquatic Animal Health 2:172–176.

Meyers, T. R., J. B. Thomas, J. E. Follett, and R. R. Saft. 1990. Infectious hematopoietic necrosis virus: Trends in prevalence and the risk management approach in Alaskan sockeye salmon culture. Journal of Aquatic Animal Health 2:85–98.

Meyers, T. R., J. Sullivan, E. Emmenegger, J. Follett, S. Short, W. N. Batts, and J. R. Winton. 1991. Isolation of viral hemorrhagic septicemia virus from Pacific cod *Gadus macrocephalus* in Prince William Sound, Alaska. Pages 83–91 [*In*] Proceedings from the 2nd International Symposium on Viruses of Lower Vertebrates, Corvallis, Oregon.

Meyers, T. R., J. Sullivan, E. Emmenegger, J. Follett, S. Short, W. N. Batts and J. R. Winton. 1992. Identification of viral hemorrhagic septicemia virus isolated from Pacific cod *Gadus macrocephalus* in Prince William Sound, Alaska, USA. Diseases of Aquatic Organisms 12:167–175.

Meyers, T. R., S. Short, C. Farrington, K. Lipson, H. J. Geiger, and R. Gates. 1993. Comparison of the enzyme-linked immunosorbent assay (ELISA) and the fluorescent antibody test (FAT) for measuring the prevalences and levels of *Renibacterium salmoninarum* in wild and hatchery stocks of salmonid fishes in Alaska, USA. Diseases of Aquatic Organisms 16:181–189.

Meyers, T. R., S. Short, C. Farrington, K. Lipson, H. J. Geiger, and R. Gates. 1993. Establishment of a negative-positive threshold optical density value for the enzyme-linked immunosorbent assay (ELISA) to detect soluble antigen of *Renibacterium salmoninarum* in Alaskan Pacific salmon. Diseases of Aquatic Organisms 16:191–197.

Meyers, T. R., S. Short, K. Lipson, W. N. Batts, J. R. Winton, J. Wilcock, and E. Brown. 1994. Association of viral hemorrhagic septicemia virus with epizootic hemorrhages of the skin in Pacific herring *Clupea harengus pallasi* from Prince William Sound and Kodiak Island, Alaska, USA. Diseases of Aquatic Organisms 19:27–37.

Meyers, T. R., D. V. Lightner, and R. M. Redman. 1994. A dinoflagellate-like parasite in Alaskan spot, *Pandalus platyceros*, and pink, *P. borealis*, shrimp. Diseases of Aquatic Organisms 18:71–76.

Meyers, T. R., J. F. Morado, A. K. Sparks, G. H. Bishop, T. Pearson, D. Urban, and D. Jackson. 1996. The distribution of bitter crab syndrome in Tanner crabs (*Chionoecetes bairdi, C. opilio*) from the Gulf of Alaska and the Bering Sea. Diseases of Aquatic Organisms 26:221–227.

Meyers, T. R. and J. R. Winton. 1996. Viral hemorrhagic septicemia virus in North America. Annual Review of Fish Diseases 5:3–24.

Meyers, T. R. 1998. Healthy juvenile sockeye salmon reared in virus-free hatchery water return as adults infected with IHNV: a case report and review of controversial issues in the epizootiology of IHNV. Journal of Aquatic Animal Health 10:172–181.

Meyers, T. R., S. Short, K. Lipson. 1999. Isolation of the North American strain of viral hemorrhagic septicemia virus (VHSV) associated with epizootic mortality in two new host species of Alaskan marine fishes. Diseases of Aquatic Organisms 38:81–86.

Meyers, T. R., F. Thrower, S. Short, K. Lipson, J. Joyce, C. Farrington, and S. Doherty. 1999. Different prevalences of *Renibacterium salmoninarum* detected by ELISA in Alaskan Chinook salmon *Oncorhynchus tshawytscha* spawned from freshwater and seawater. Diseases of Aquatic Organisms 35:101–105.

Meyers, T. R., editor. 2000. Fish pathology section laboratory manual. Special Publications No. 12, Alaska Department of Fish and Game, 2nd edition, Commercial Fisheries Division, Juneau, Alaska.

Meyers, T. R., D. Korn, T. M. Burton, K. Glass, J. E. Follett, J. B. Thomas, J. Sullivan, N. Starkey, S. Short, and K. Lipson. 2003. IHNV in Alaskan sockeye salmon culture from 1973 to 2000: Annual virus prevalences and titers in brood-stocks compared to juvenile losses. Journal of Aquatic Animal Health 15:21–30.

Meyers, T. R., D. Korn, K. Glass, T. M. Burton, S. Short, K. Lipson, and N. Starkey. 2003. Retrospective analysis of antigen prevalences of *Renibacterium salmoninarum* (Rs) detected by ELISA in Alaskan Pacific salmon and trout from 1988 to 2000. Journal of Aquatic Animal Health 15:101–110.

Meyers, T. R. 2004. Standard necropsy procedures for finfish. *In* K. True, editor. National wild fish health survey-laboratory procedures manual. 2nd Edition, U.S. Fish and Wildlife Service, Anderson, California.

Meyers, T. R. 2004. Disease transmission from cultured salmonids to wild fish stocks: perspectives on the Alaskan hatchery program. [*In*] R. C. Cipriano and I. S. Shchelkunov, editors. Health and diseases of aquatic organisms: bilateral perspectives. Michigan University Press.

Meyers, T. R. 2005. Marteiliasis and Marteilioidiasis of shellfish. 5.2.9 [*In*] Fish Health Section Bluebook. http://afs-fhs.org/bluebook/bluebook-index.php

Meyers, T. R. 2007. First report of erythrocytic inclusion body syndrome (EIBS) in Chinook salmon *Oncorhynchus tshawytscha* in Alaska, USA. Diseases of Aquatic Organisms 76:169–172.

Meyers, T. R., T. Burton, C. Bentz, and N. Starkey. 2008. Common diseases of wild and cultured fishes in Alaska. Alaska Department of Fish and Game, Division of Commercial Fisheries, Anchorage Alaska.

Meyers, T. R., and T. Burton. 2009. Diseases of wild and cultured shellfish in Alaska. Alaska Department of Fish and Game, Division of Commercial Fisheries, Anchorage, Alaska.

Meyers, T. R., editor. 2009. Fish pathology section laboratory manual. Special Publications No. 12, 3rd edition. Alaska Department of Fish and Game, Division of Commercial Fisheries, Juneau, Alaska.

Meyers, T. R., T. Burton, W. Evans, N. Starkey. 2009. Detection of viruses and virus-like particles in four species of wild and farmed bivalve molluscs in Alaska, USA from 1987 to 2009. Diseases of Aquatic Organisms 88:1–12.

Meyers, T. 2010. Regulation changes, policies and guidelines for Alaska fish and shellfish health and disease control. Alaska Department of Fish and Game, Regional Report Series No. 5J10-01, Juneau, Alaska.

Meyers, T. R. 2014. Infection with *Hematodinium*. 6.2.5 [*In*] Fish Health Section Bluebook. http://afs-fhs.org/bluebook/bluebook-index.php

Oshima, K. H., K. H. Higman, C. K. Arakawa, P. de Kinkelin, P. E. Vestergard Jorgensen, T. R. Meyers, and J. R. Winton. 1993. Genetic comparison of viral hemorrhagic septicemia virus isolates from North America and Europe. Diseases of Aquatic Organisms. 17:73–80.

Rutecki, L., and T. R. Meyers. 1992. Mortality of juvenile sablefish captured by hand-jigging and traps. North American Journal of Fisheries Management 12:836–837.

Weiland, K. A., and T. R. Meyers. 1989. Histopathology of *Diphyllobothrium ditremum* plerocercoids in coho salmon *Oncorhynchus kisutch*. Diseases of Aquatic Organisms 6:175–178.

Winton, J. R., C. N. Lannan, J. L. Fryer, R. P. Hedrick, T. R. Meyers, J. A. Plumb, and T. Yamamoto. 1987. Morphological and biochemical properties of four members of a novel group of reoviruses isolated from aquatic animals. Journal of General Virology 68:353–364.